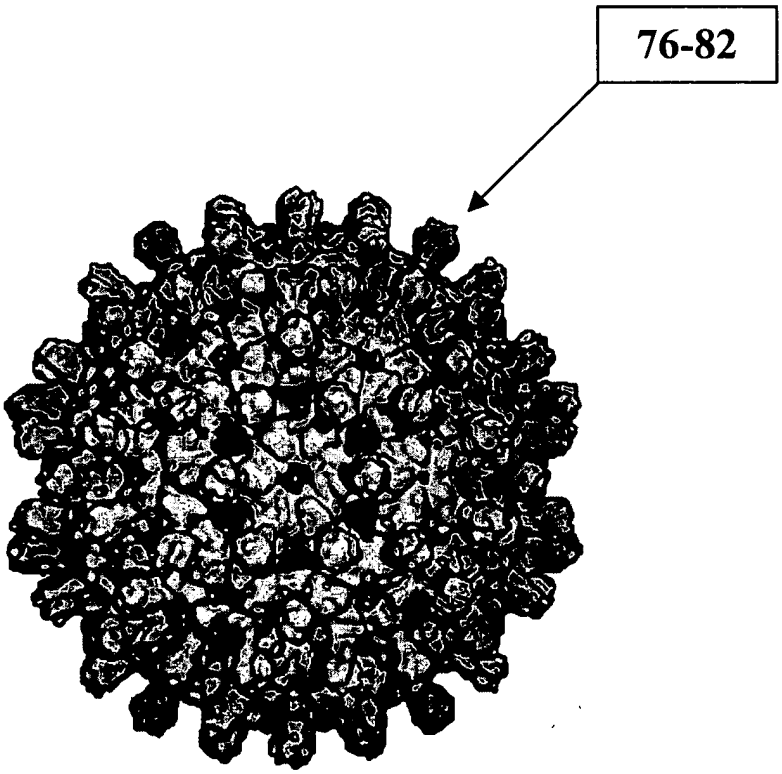


Fig. 1

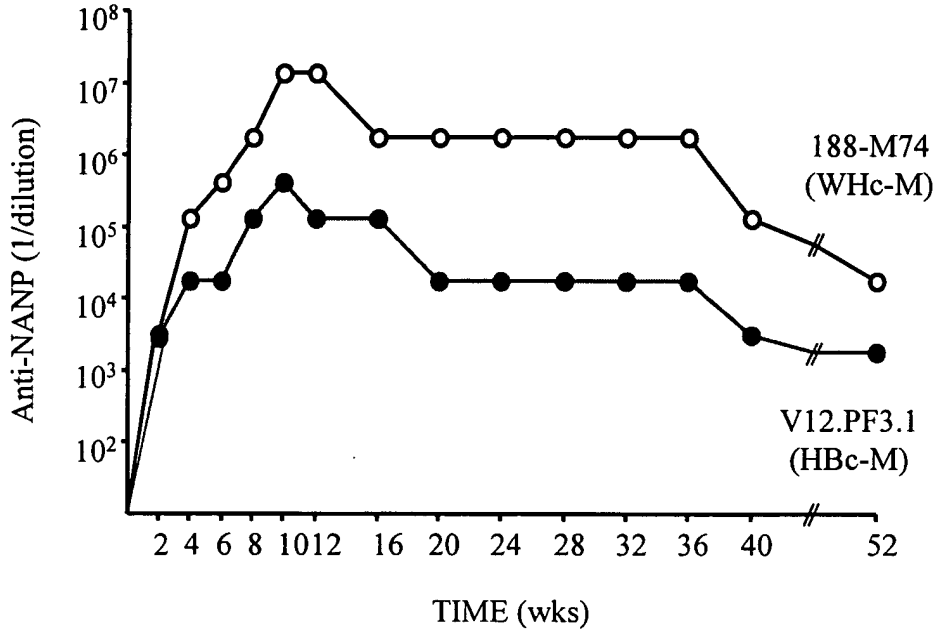
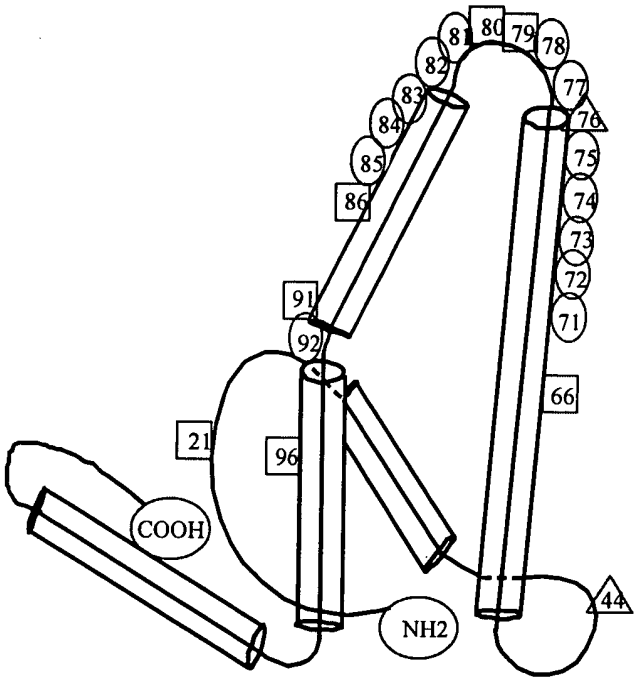

Anti-NANP (1/dilution)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
188-M74
(WHc-M)
V12.PF3.1
(HBc-M)
2 4 6 8 10 12 16 20 24 28 32 36 40 52
TIME (wks)

Fig. 2



tolerant

intermediate

intolerant

Fig. 3

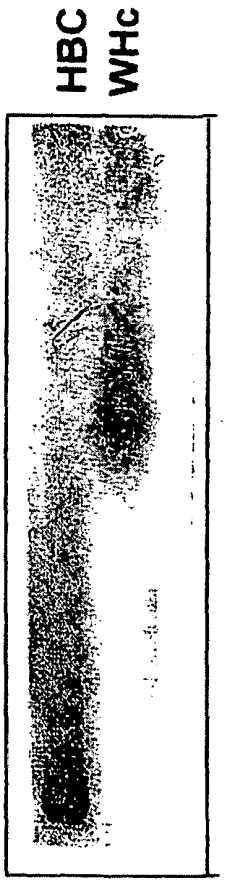


Fig. 4

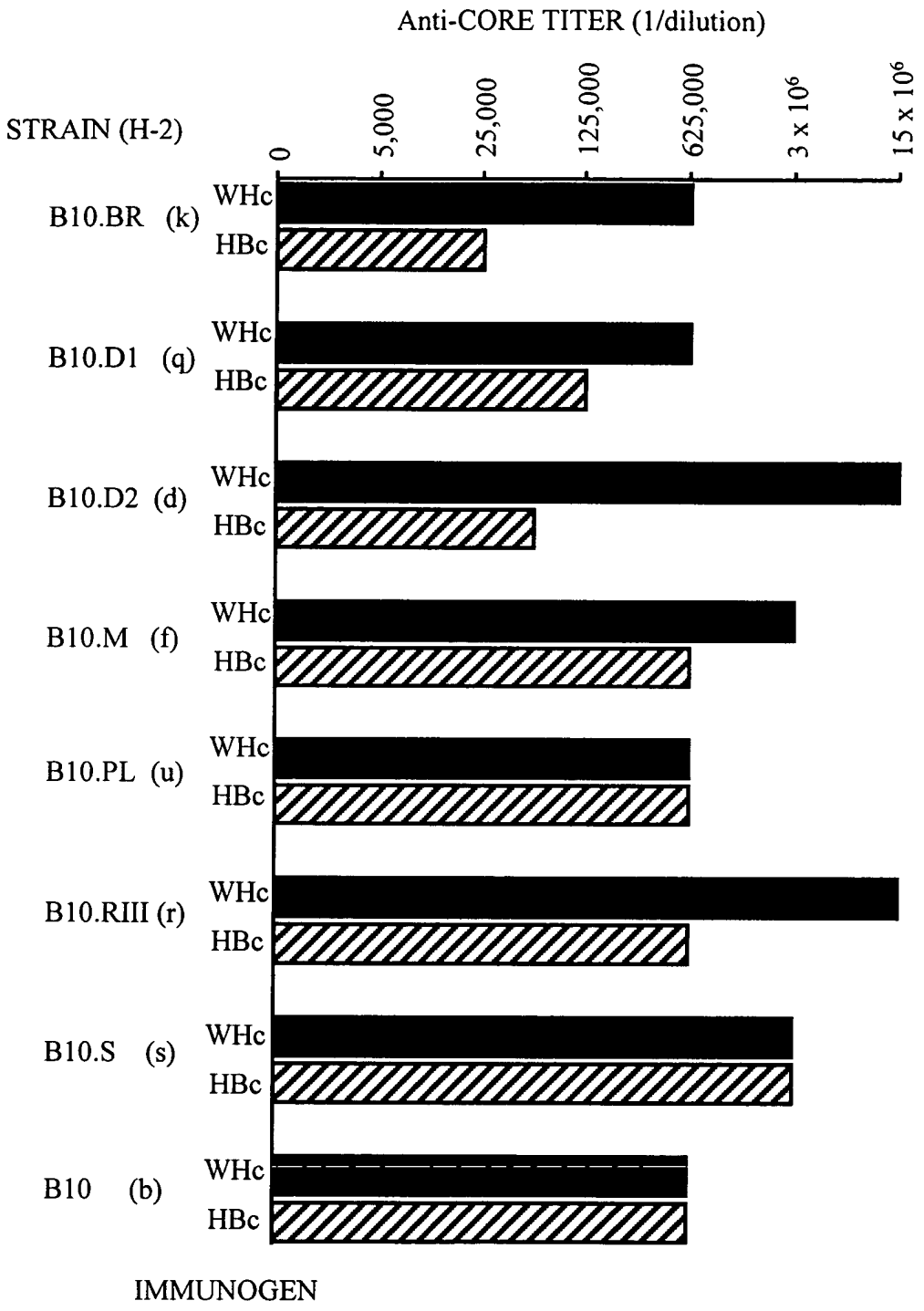

Anti-CORE TITER (1/dilution)
STRAIN (H-2)
0
5,000
25,000
125,000
625,000
3 x 10^6
15 x 10^6
B10.BR (k)
B10.D1 (q)
B10.D2 (d)
B10.M (f)
B10.PL (u)
B10.RIII (r)
B10.S (s)
B10 (b)
WHc
HBc
IMMUNOGEN

Fig. 5

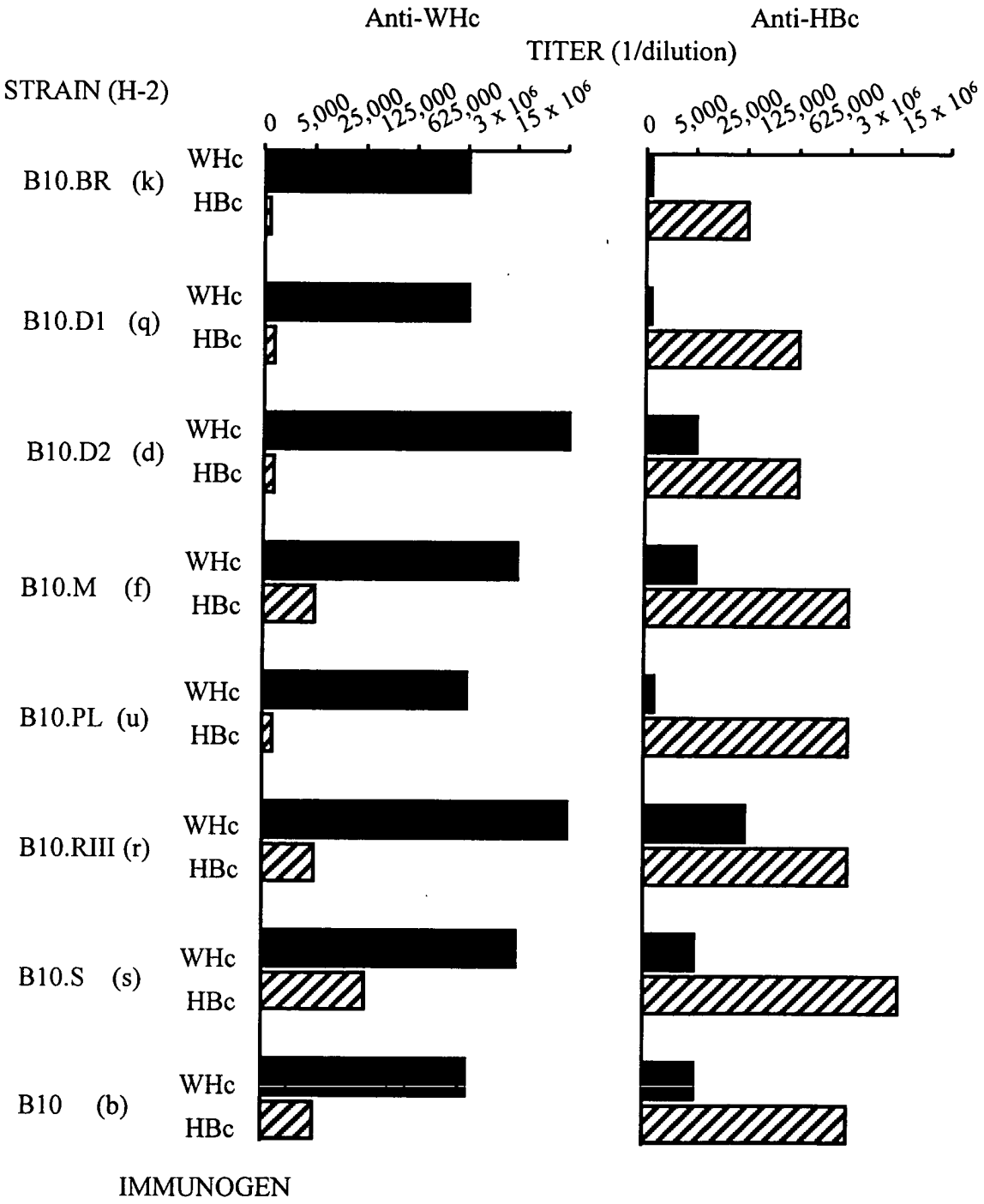

Anti-WHc
Anti-HBc
TITER (1/dilution)
STRAIN (H-2)
0 5,000 25,000 125,000 625,000 3 x 10⁶ 15 x 10⁶
B10.BR (k)
B10.D1 (q)
B10.D2 (d)
B10.M (f)
B10.PL (u)
B10.RIII (r)
B10.S (s)
B10 (b)
WHc
HBc
IMMUNOGEN

Fig. 6

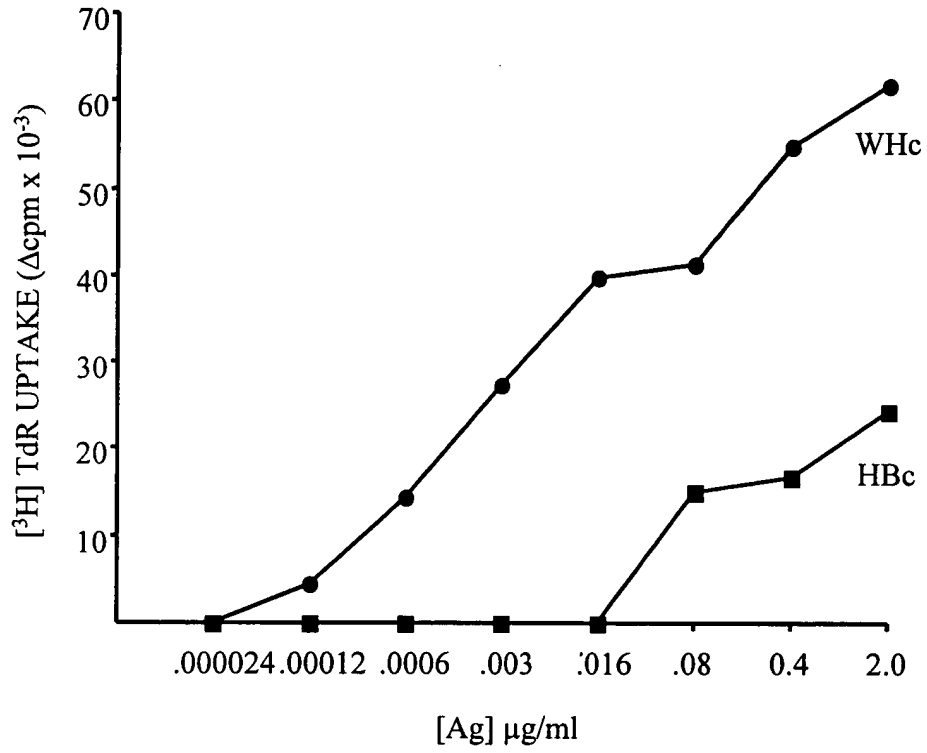

70
60
50
40
30
20
10
[3H] TdR UPTAKE (Δcpm x 10-3)
WHc
HBc
.000024
.00012
.0006
.003
.016
.08
0.4
2.0
[Ag] µg/ml

Fig. 7

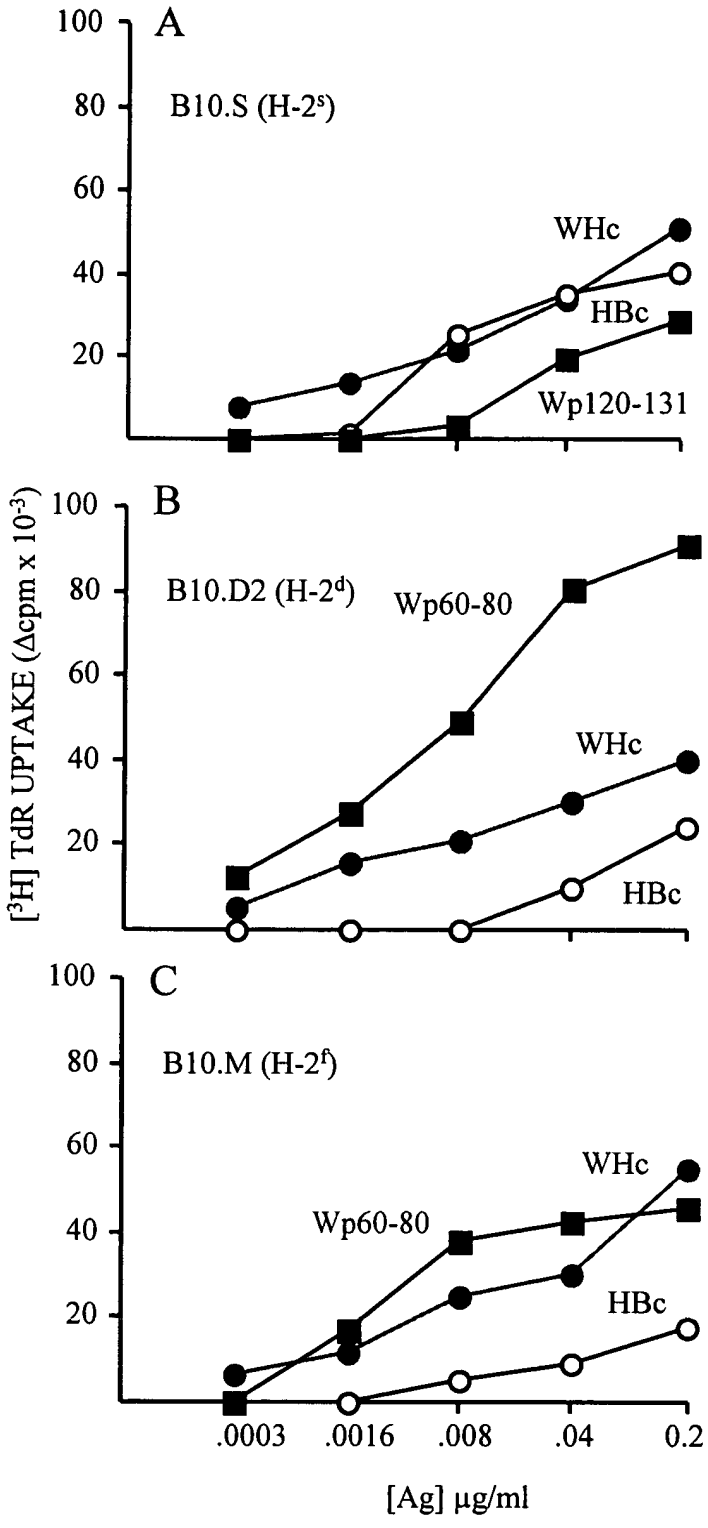

A
B10.S (H-2s)
WHc
HBc
Wp120-131
B
B10.D2 (H-2d)
Wp60-80
WHc
HBc
C
B10.M (H-2f)
WHc
Wp60-80
HBc
100
80
60
40
20
.0003
.0016
.008
.04
0.2
[3H] TdR UPTAKE (Δcpm x 10-3)
[Ag] µg/ml

Fig. 8

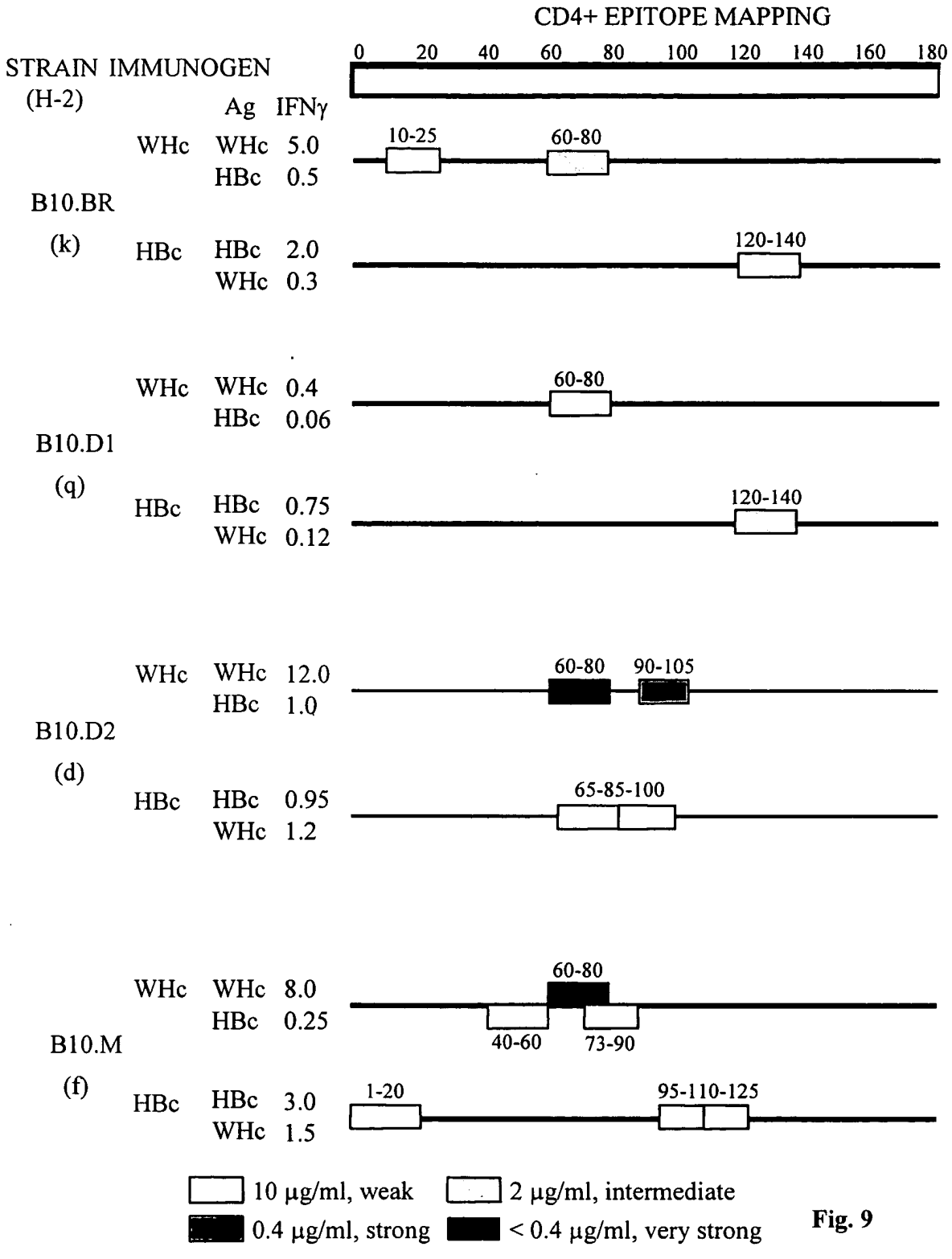

CD4+ EPITOPE MAPPING
STRAIN IMMUNOGEN
(H-2)
Ag IFNγ
0 20 40 60 80 100 120 140 160 180
WHc WHc 5.0
HBc 0.5
10-25
60-80
B10.BR
(k)
HBc HBc 2.0
WHc 0.3
120-140
WHc WHc 0.4
HBc 0.06
60-80
B10.D1
(q)
HBc HBc 0.75
WHc 0.12
120-140
WHc WHc 12.0
HBc 1.0
60-80
90-105
B10.D2
(d)
HBc HBc 0.95
WHc 1.2
65-85-100
WHc WHc 8.0
HBc 0.25
60-80
40-60
73-90
B10.M
(f)
HBc HBc 3.0
WHc 1.5
1-20
95-110-125
10 μg/ml, weak
2 μg/ml, intermediate
0.4 μg/ml, strong
< 0.4 μg/ml, very strong

Fig. 9

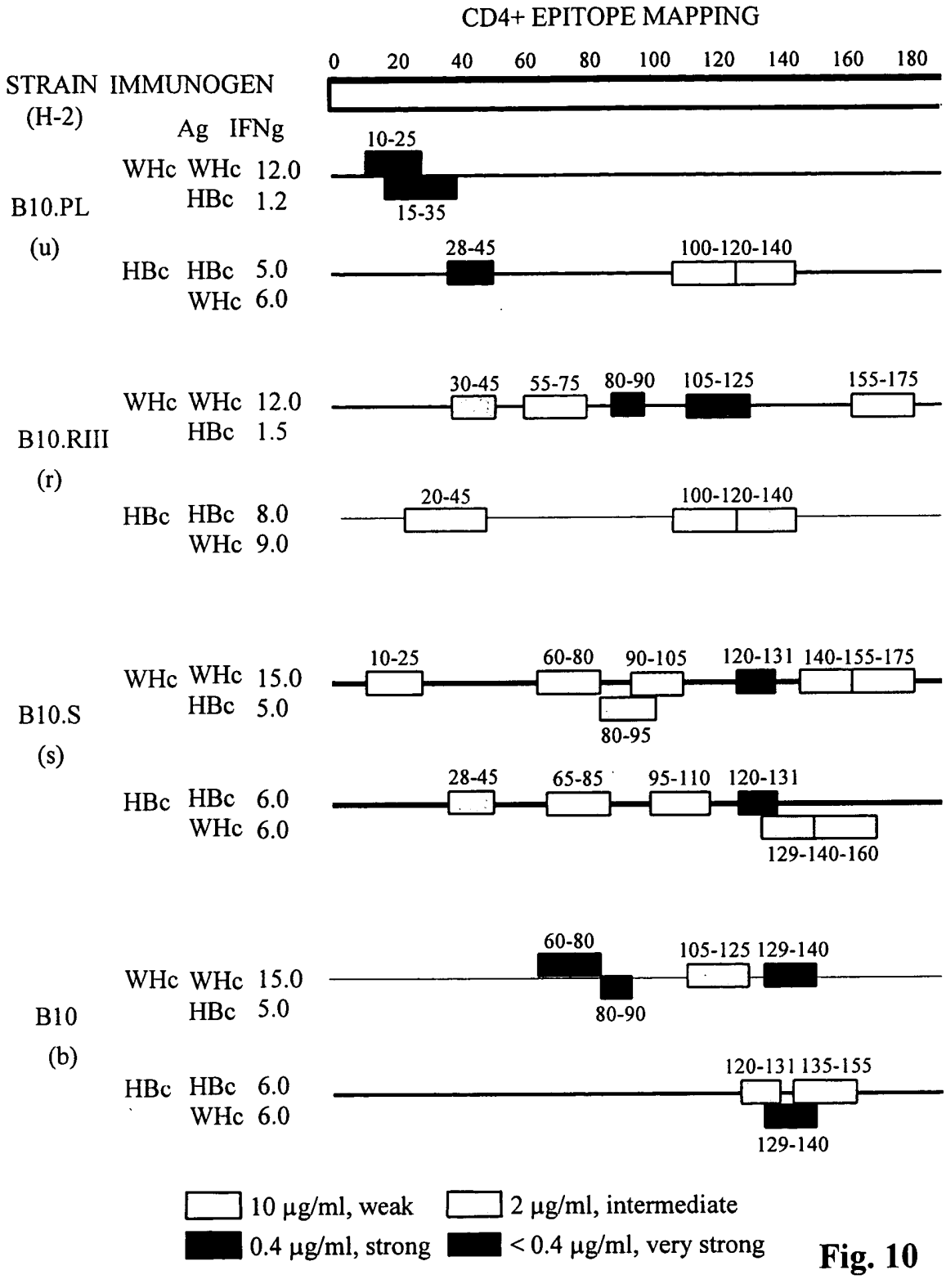

CD4+ EPITOPE MAPPING
0 20 40 60 80 100 120 140 160 180
STRAIN IMMUNOGEN
(H-2)
Ag IFNg
WHc WHc 12.0
HBc 1.2
B10.PL
(u)
10-25
15-35
HBc HBc 5.0
WHc 6.0
28-45
100-120-140
WHc WHc 12.0
HBc 1.5
B10.RIII
(r)
30-45
55-75
80-90
105-125
155-175
HBc HBc 8.0
WHc 9.0
20-45
100-120-140
WHc WHc 15.0
HBc 5.0
B10.S
(s)
10-25
60-80
90-105
120-131
140-155-175
80-95
HBc HBc 6.0
WHc 6.0
28-45
65-85
95-110
120-131
129-140-160
WHc WHc 15.0
HBc 5.0
B10
(b)
60-80
105-125
129-140
80-90
HBc HBc 6.0
WHc 6.0
120-131 135-155
129-140
10 µg/ml, weak
2 µg/ml, intermediate
0.4 µg/ml, strong
< 0.4 µg/ml, very strong

Fig. 10

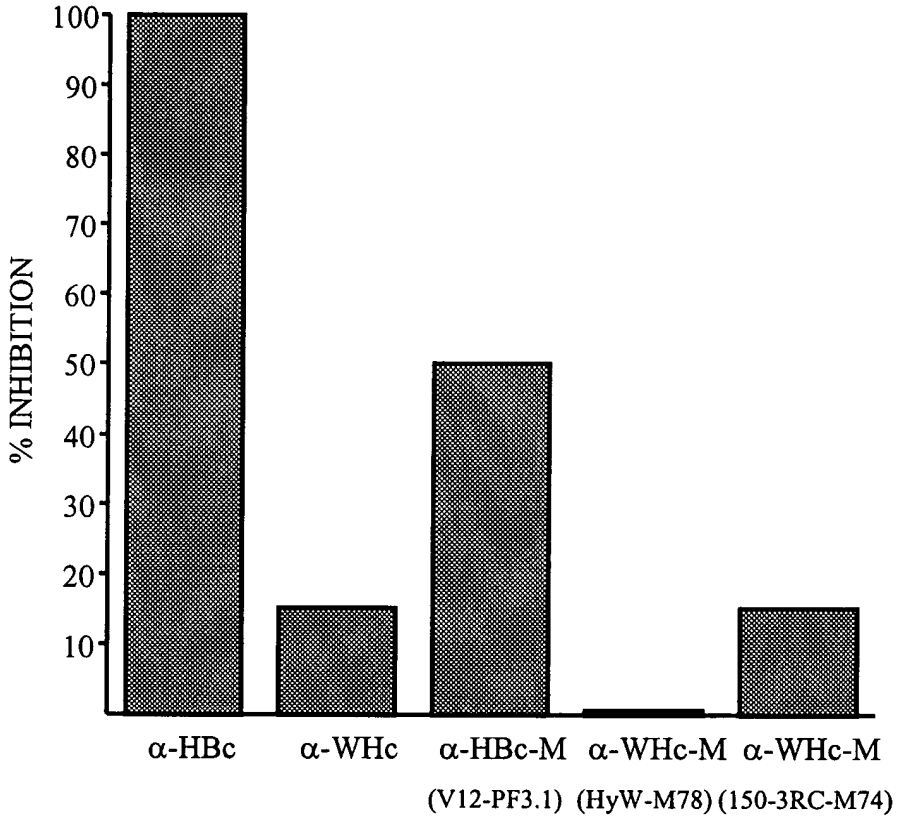

100
90
80
70
60
50
40
30
20
10
% INHIBITION
α-HBc
α-WHc
α-HBc-M (V12-PF3.1)
α-WHc-M (HyW-M78)
α-WHc-M (150-3RC-M74)
Murine Antisera (1:10)

Fig. 11

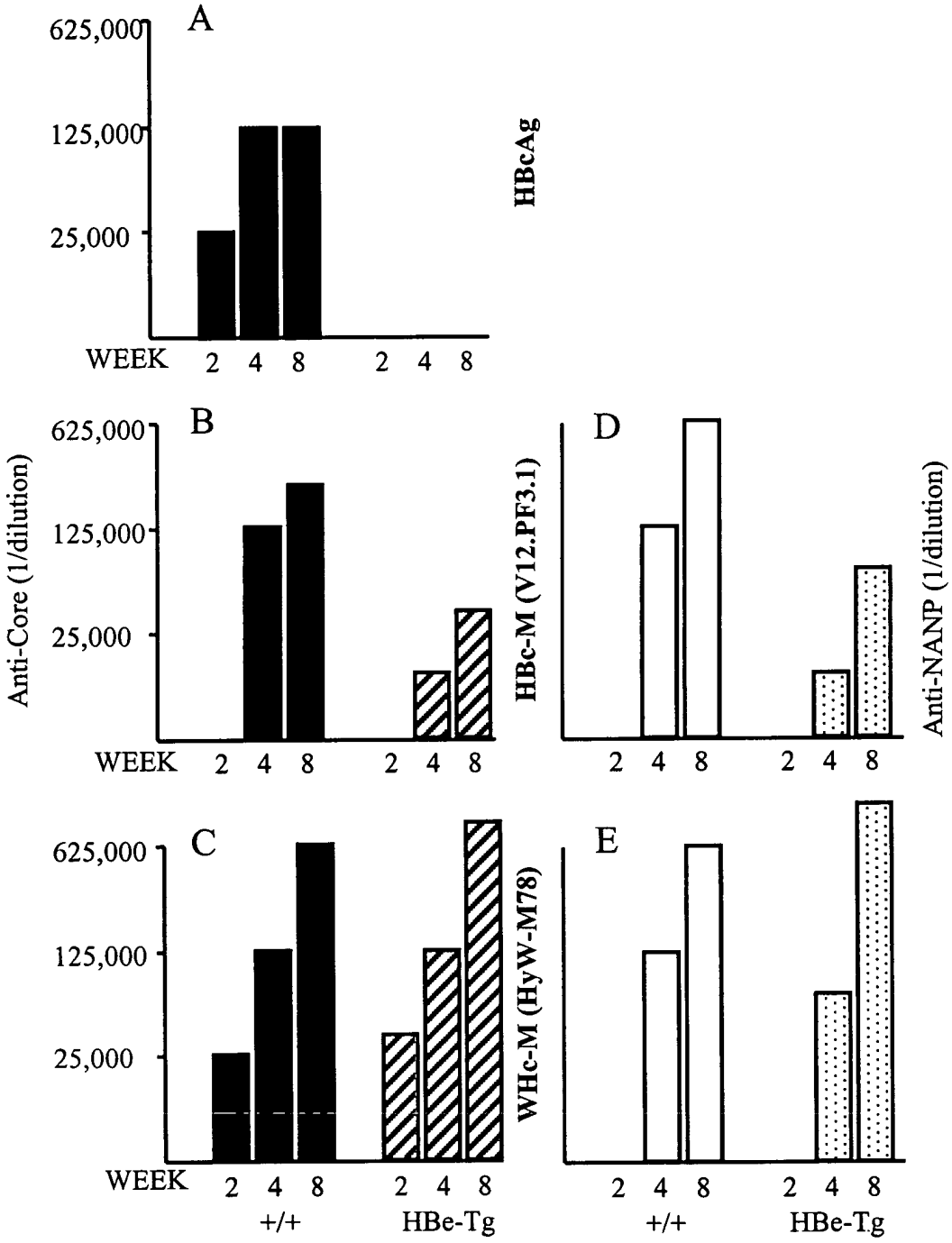

A
625,000
125,000
25,000
HBcAg
WEEK 2 4 8 2 4 8
B
625,000
125,000
25,000
Anti-Core (1/dilution)
WEEK 2 4 8 2 4 8
D
HBc-M (V12.PF3.1)
Anti-NANP (1/dilution)
2 4 8 2 4 8
C
625,000
125,000
25,000
WHc-M (HyW-M78)
WEEK 2 4 8 2 4 8
+/+ HBe-Tg
E
2 4 8 2 4 8
+/+ HBe-Tg

Fig. 12

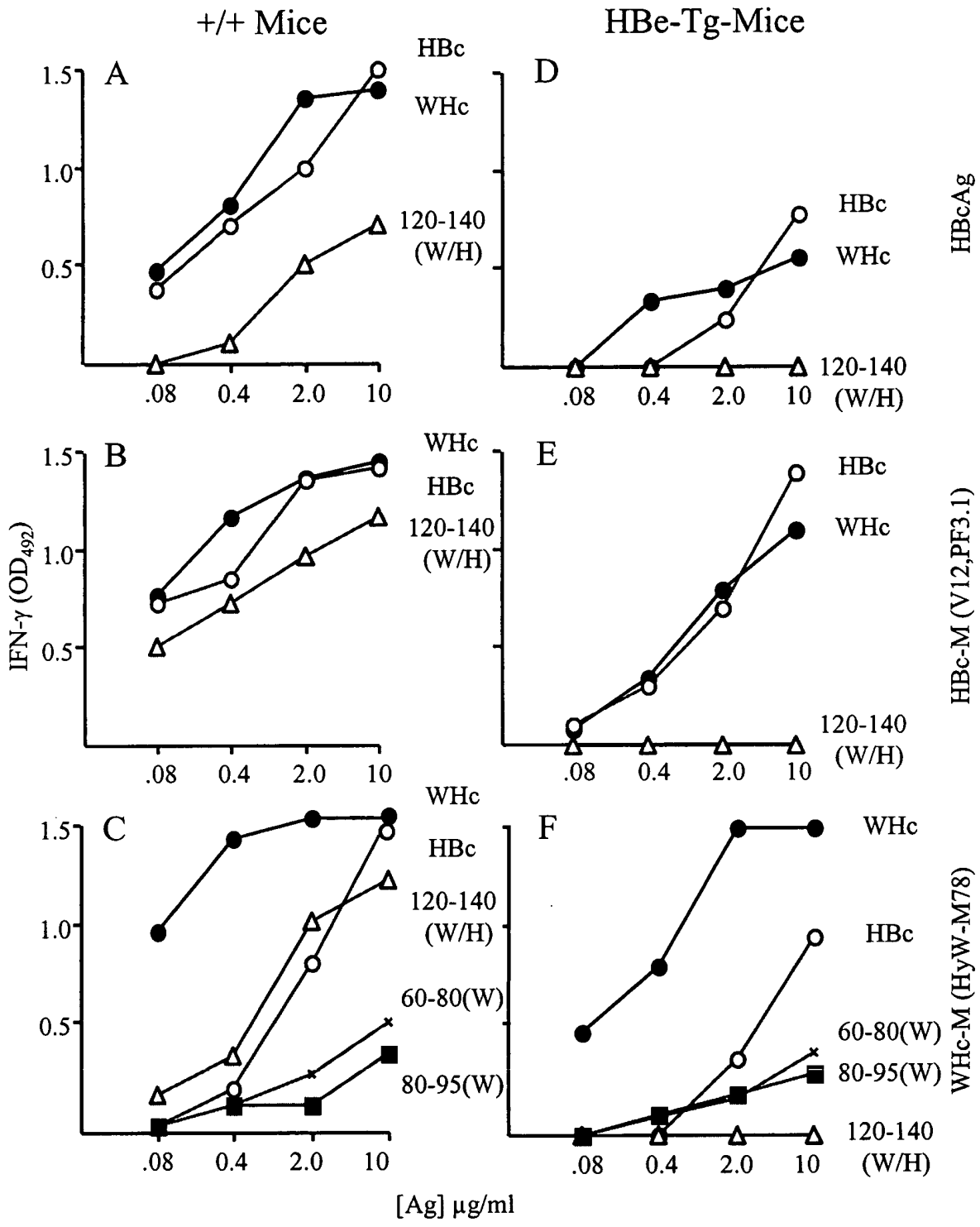
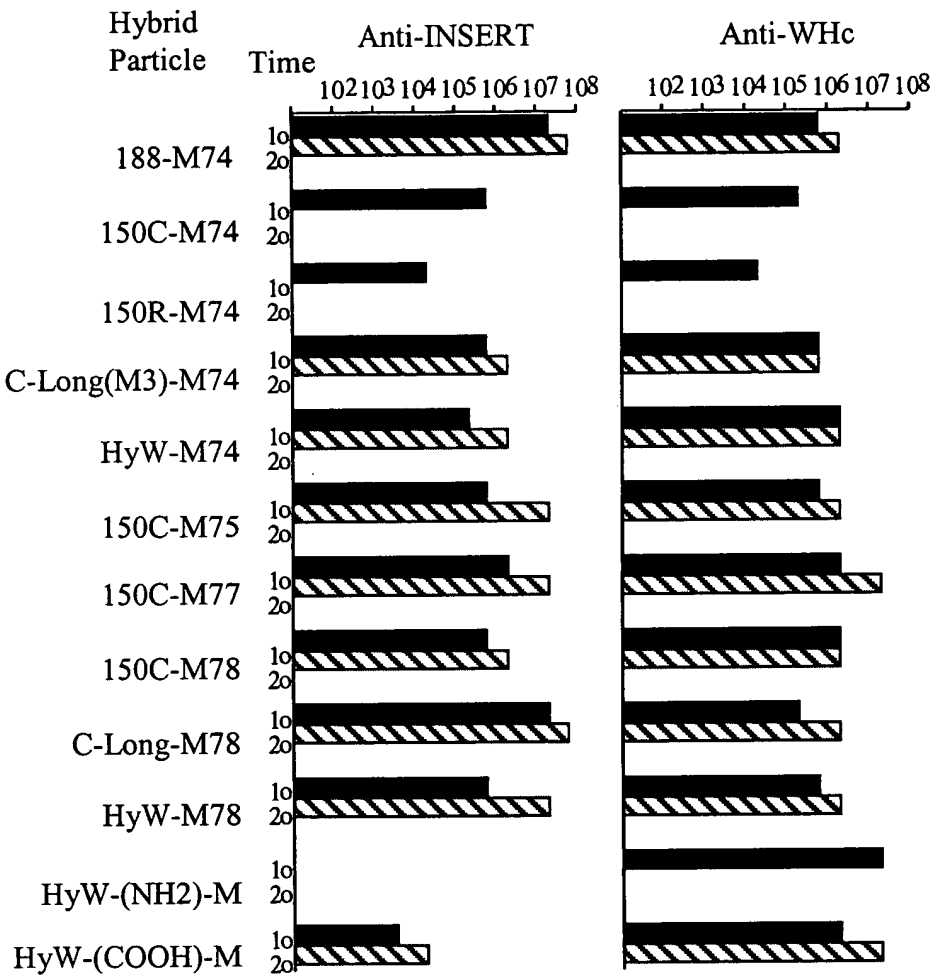

+/+ Mice
HBe-Tg-Mice
A
B
C
D
E
F
HBc
WHc
120-140 (W/H)
60-80(W)
80-95(W)
1.5
1.0
0.5
.08
0.4
2.0
10
IFN-γ (OD492)
HBcAg
HBc-M (V12,PF3.1)
WHc-M (HyW-M78)
[Ag] µg/ml

Fig. 13



IMMUNOGENICITY

Fig. 14

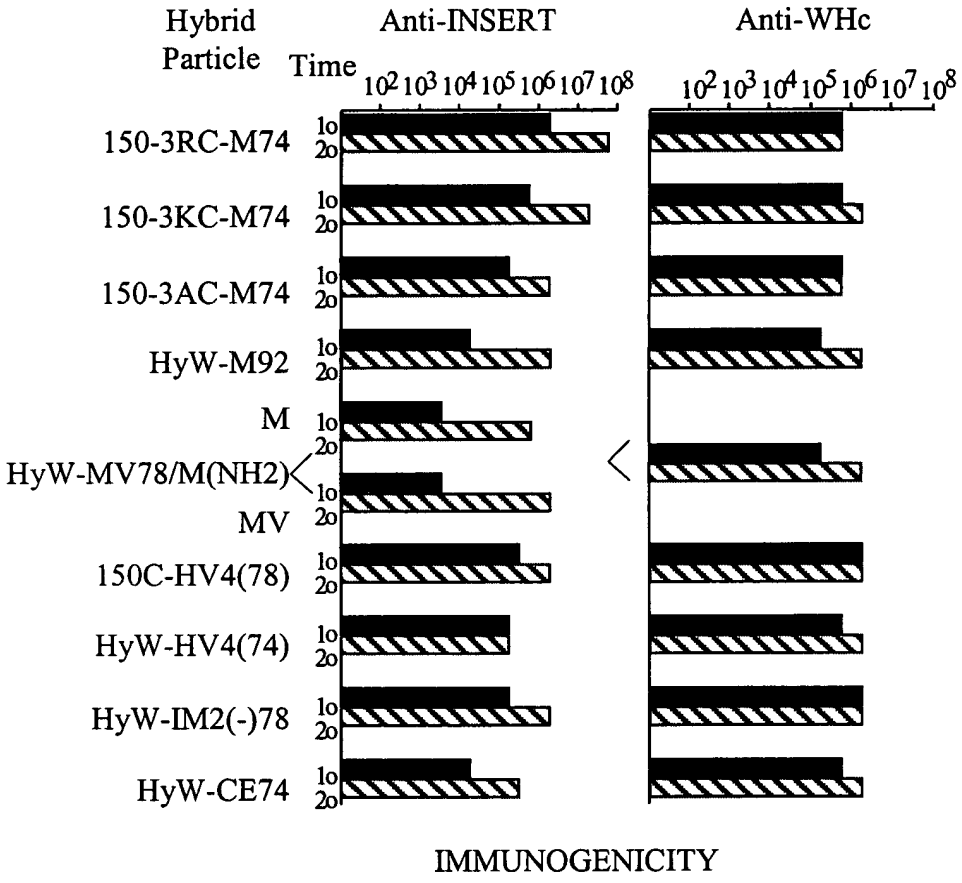

Hybrid Particle
Time
Anti-INSERT
Anti-WHc
10^2 10^3 10^4 10^5 10^6 10^7 10^8
150-3RC-M74
150-3KC-M74
150-3AC-M74
HyW-M92
M
HyW-MV78/M(NH2)
MV
150C-HV4(78)
HyW-HV4(74)
HyW-IM2(-)78
HyW-CE74
1o
2o
IMMUNOGENICITY

Fig. 15

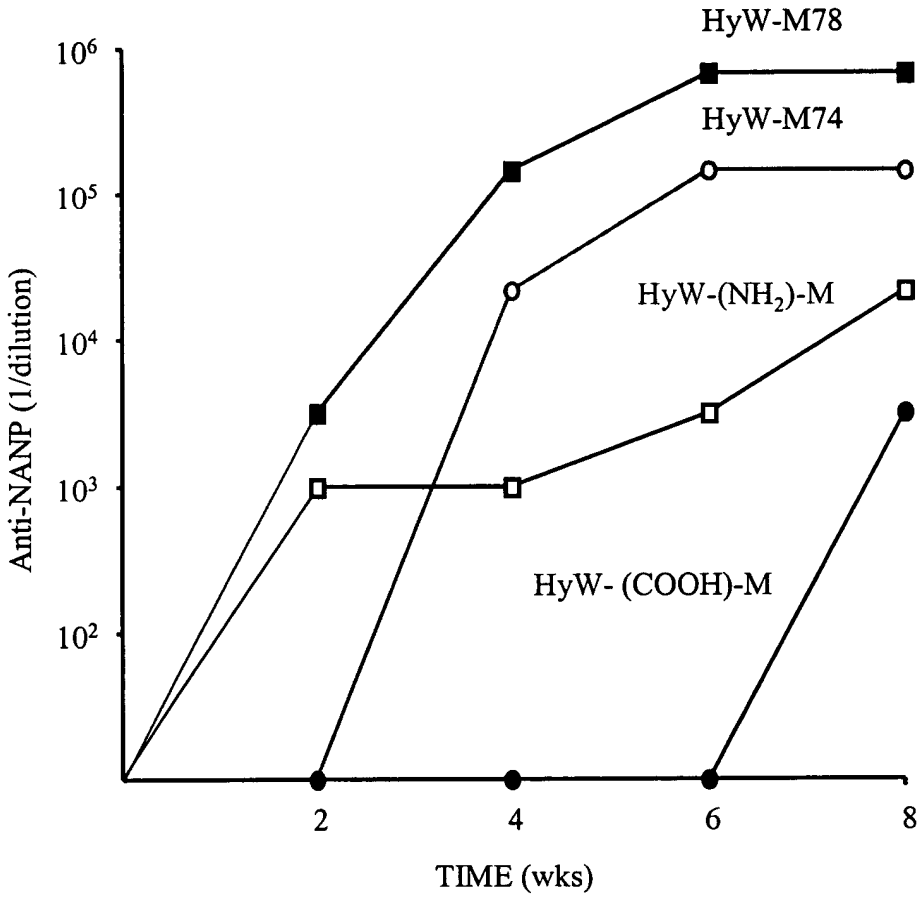

HyW-M78
HyW-M74
HyW-(NH2)-M
HyW- (COOH)-M
Anti-NANP (1/dilution)
10^6
10^5
10^4
10^3
10^2
2
4
6
8
TIME (wks)

Fig. 16

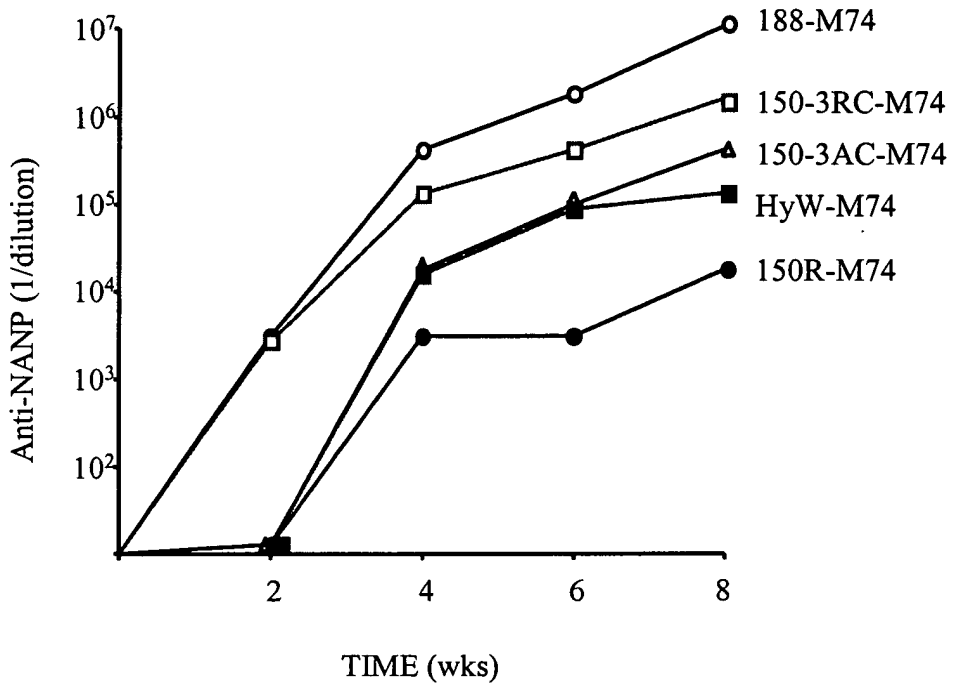

Anti-NANP (1/dilution)
10^7
10^6
10^5
10^4
10^3
10^2
2
4
6
8
TIME (wks)
188-M74
150-3RC-M74
150-3AC-M74
HyW-M74
150R-M74

Fig. 17

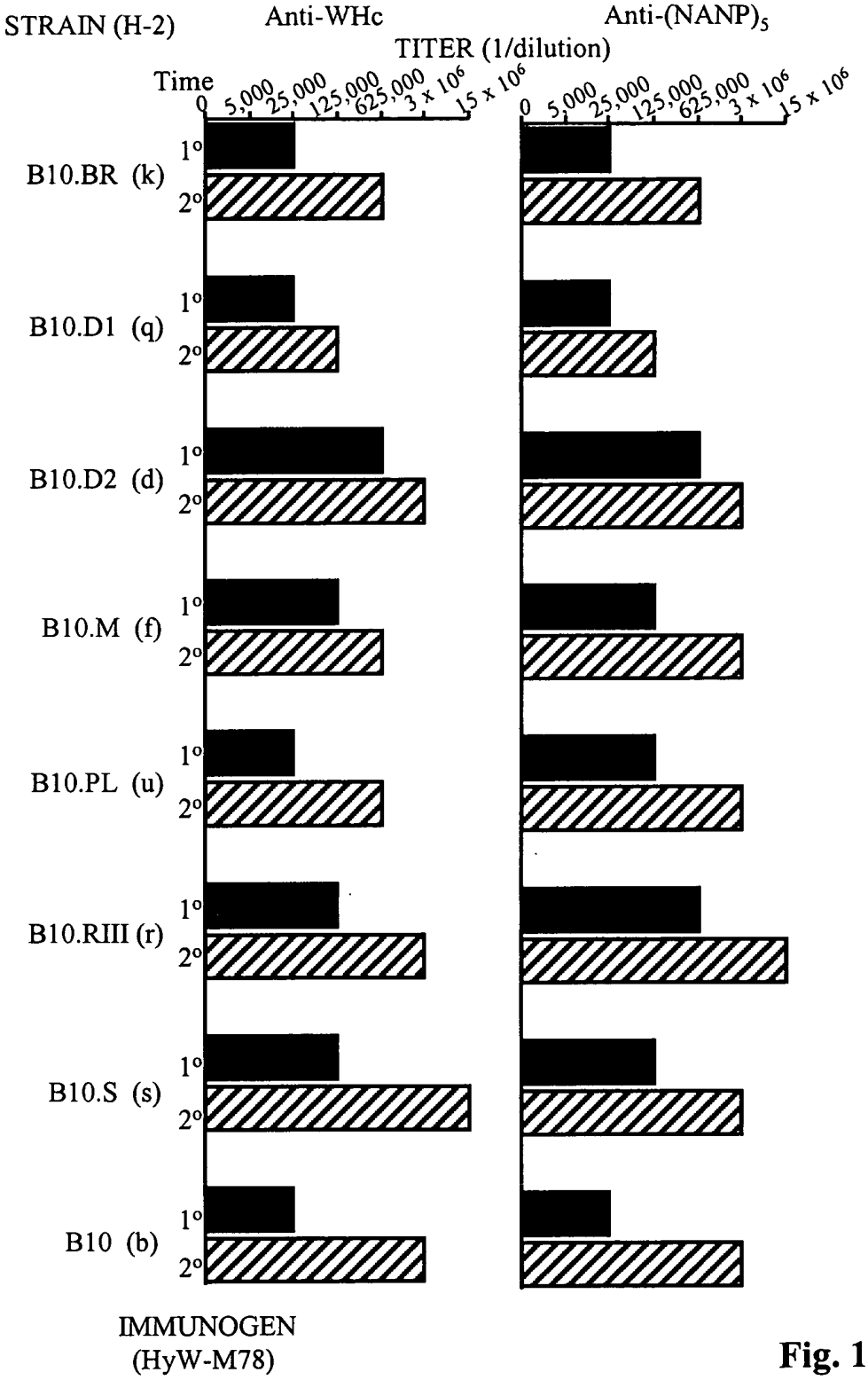

STRAIN (H-2)
Anti-WHc
Anti-(NANP)5
TITER (1/dilution)
Time
0 5,000 25,000 125,000 625,000 3 x 10^6 15 x 10^6
0 5,000 25,000 125,000 625,000 3 x 10^6 15 x 10^6
B10.BR (k)
B10.D1 (q)
B10.D2 (d)
B10.M (f)
B10.PL (u)
B10.RIII (r)
B10.S (s)
B10 (b)
1°
2°
IMMUNOGEN
(HyW-M78)

Fig. 18

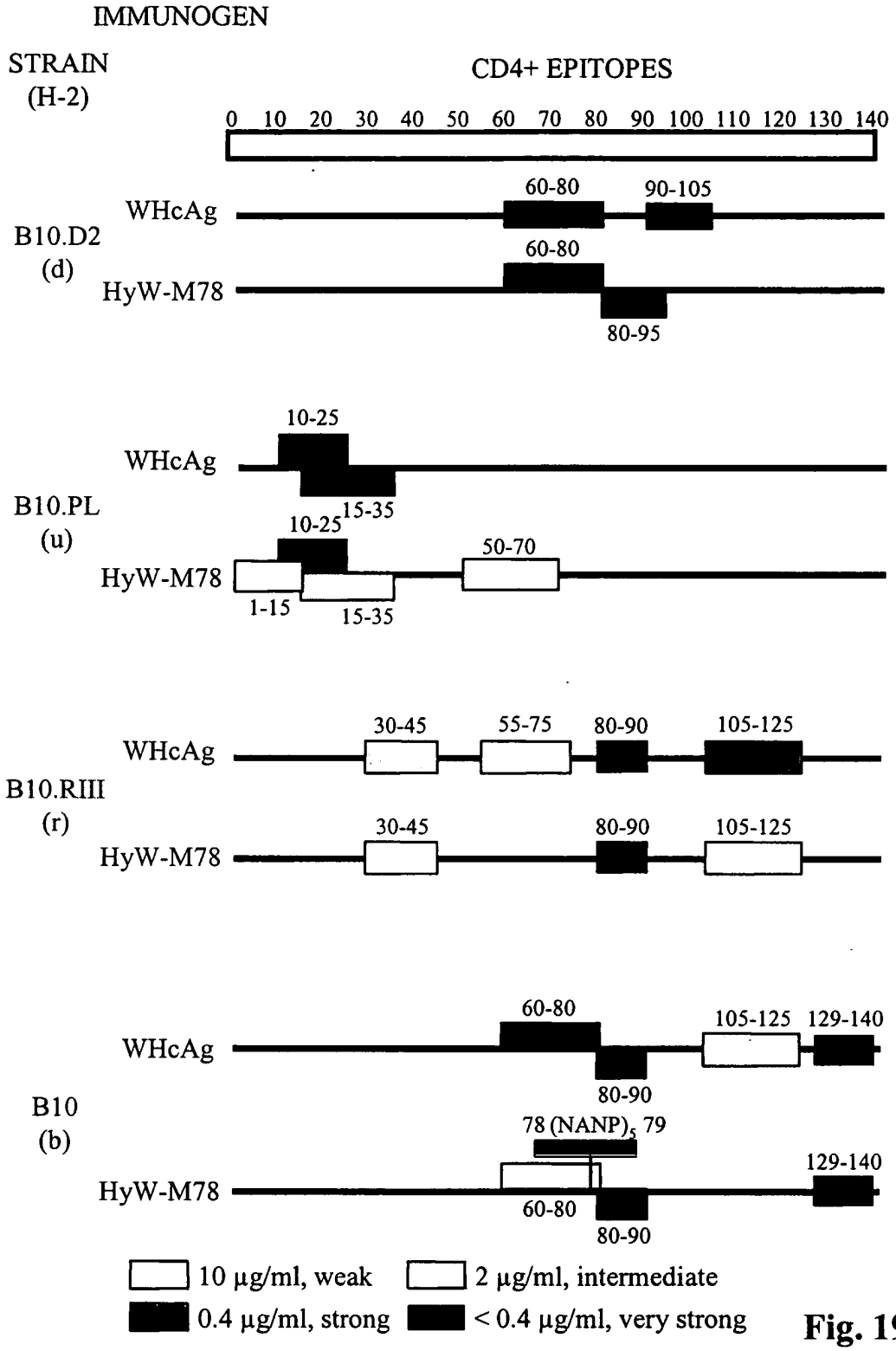

IMMUNOGEN
STRAIN
(H-2)
CD4+ EPITOPES
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
B10.D2
(d)
WHcAg
60-80
90-105
HyW-M78
60-80
80-95
B10.PL
(u)
WHcAg
10-25
15-35
HyW-M78
10-25
50-70
1-15
15-35
B10.RIII
(r)
WHcAg
30-45
55-75
80-90
105-125
HyW-M78
30-45
80-90
105-125
B10
(b)
WHcAg
60-80
105-125
129-140
80-90
HyW-M78
78 (NANP)5 79
129-140
60-80
80-90
10 µg/ml, weak
2 µg/ml, intermediate
0.4 µg/ml, strong
< 0.4 µg/ml, very strong

Fig. 19

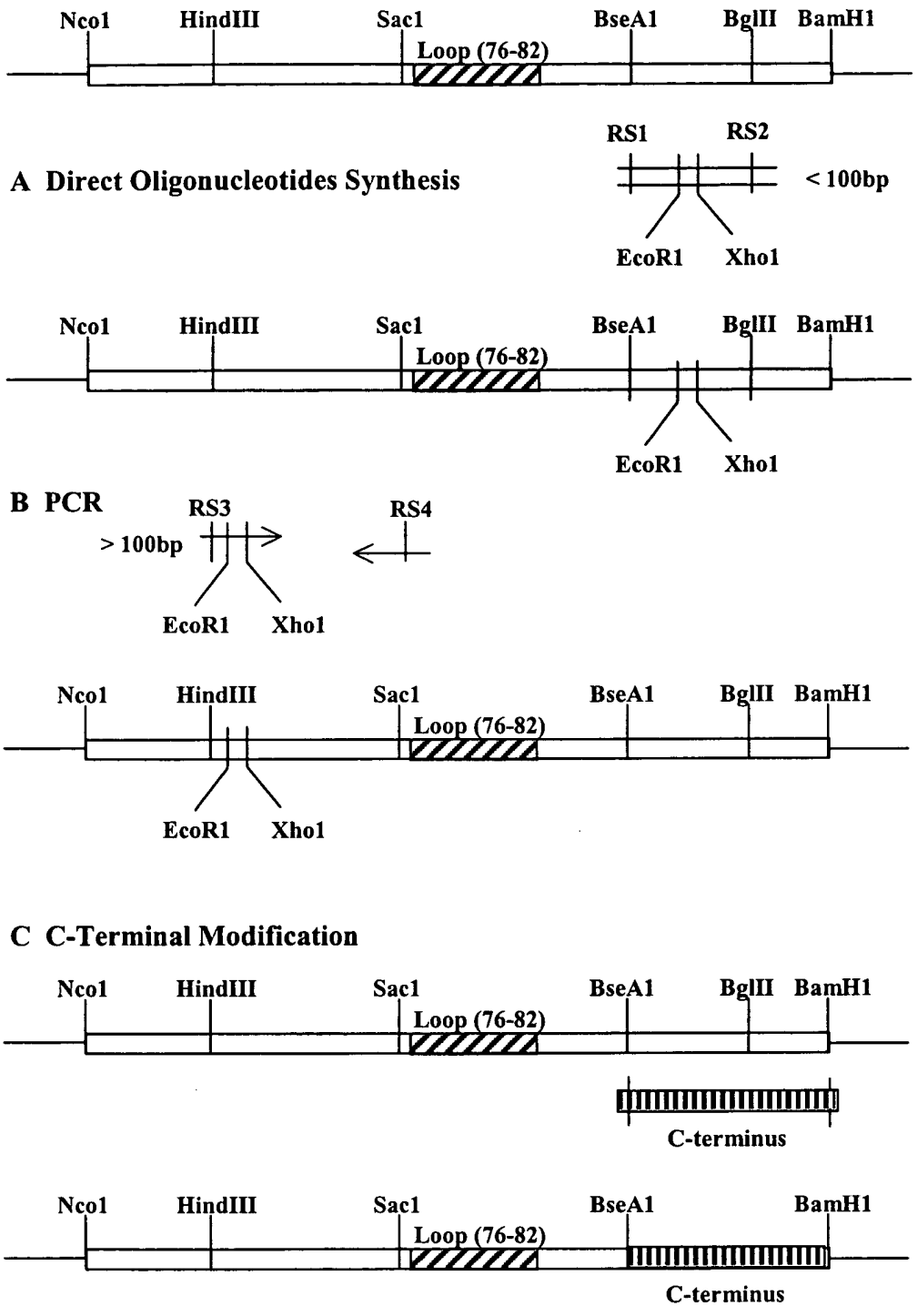

Nco1
HindIII
Sac1
Loop (76-82)
BseA1
BglII
BamH1
A Direct Oligonucleotides Synthesis
RS1
RS2
< 100bp
EcoR1
Xho1
B PCR
RS3
RS4
> 100bp
C C-Terminal Modification
C-terminus

Fig. 20

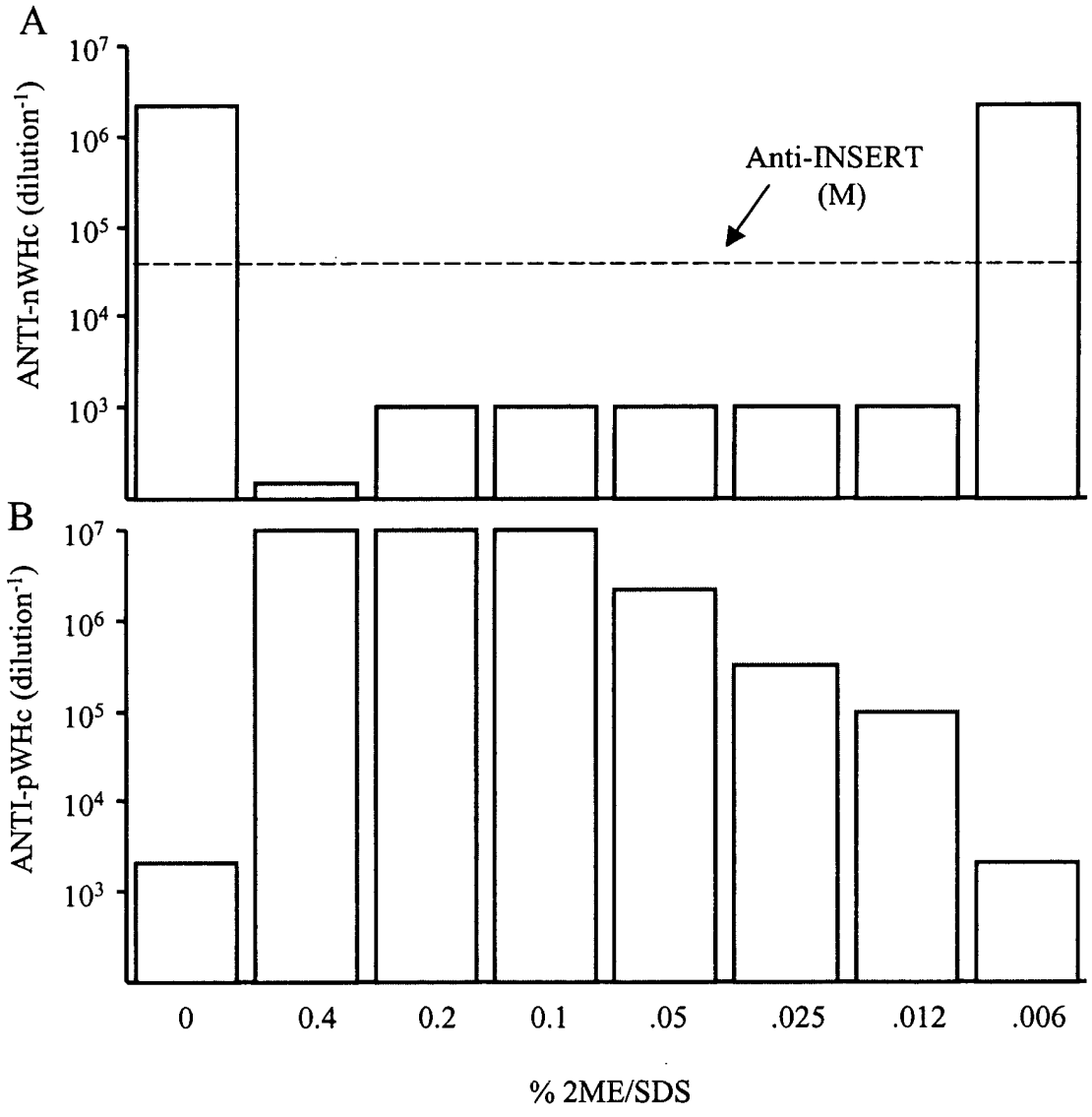

A
ANTI-nWHc (dilution-1)
10^7
10^6
10^5
10^4
10^3
Anti-INSERT
(M)
B
ANTI-pWHc (dilution-1)
10^7
10^6
10^5
10^4
10^3
0
0.4
0.2
0.1
.05
.025
.012
.006
% 2ME/SDS

Fig. 21

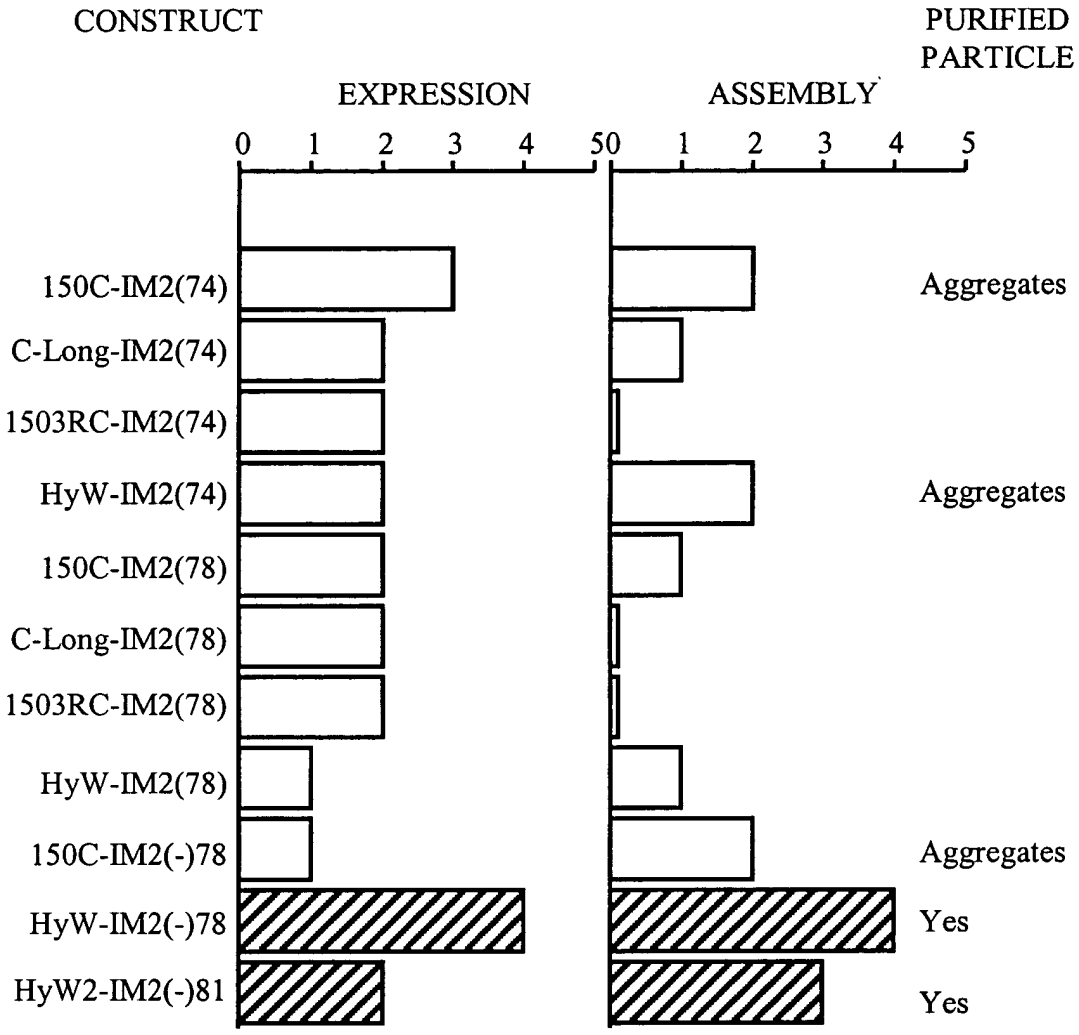

CONSTRUCT
PURIFIED PARTICLE
EXPRESSION
ASSEMBLY
0 1 2 3 4 50 1 2 3 4 5
150C-IM2(74)
C-Long-IM2(74)
1503RC-IM2(74)
HyW-IM2(74)
150C-IM2(78)
C-Long-IM2(78)
1503RC-IM2(78)
HyW-IM2(78)
150C-IM2(-)78
HyW-IM2(-)78
HyW2-IM2(-)81
Aggregates
Aggregates
Aggregates
Yes
Yes

Fig. 22

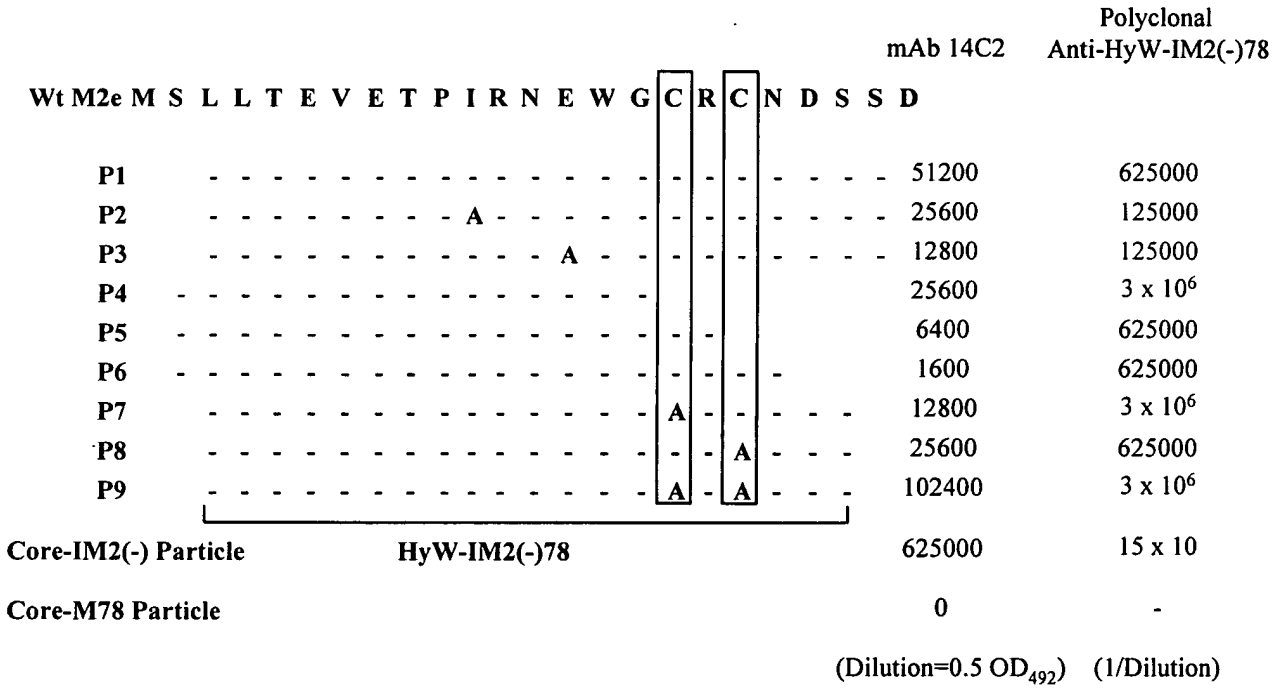

mAb 14C2
Polyclonal Anti-HyW-IM2(-)78
Wt M2e M S L L T E V E T P I R N E W G C R C N D S S D
P1 - 51200 625000
P2 - - - - - - - - - A - - - - - - - - - - - - - 25600 125000
P3 - - - - - - - - - - - - A - - - - - - - - - - 12800 125000
P4 - - - - - - - - - - - - - - - - 25600 3 x 10^6
P5 - - - - - - - - - - - - - - - - - - 6400 625000
P6 - 1600 625000
P7 - - - - - - - - - - - - - - - - A - - - - - 12800 3 x 10^6
P8 - - - - - - - - - - - - - - - - - - A - - - 25600 625000
P9 - - - - - - - - - - - - - - - - A - A - - - 102400 3 x 10^6
Core-IM2(-) Particle
HyW-IM2(-)78
625000 15 x 10
Core-M78 Particle
0 -
(Dilution=0.5 OD492) (1/Dilution)

Fig. 23

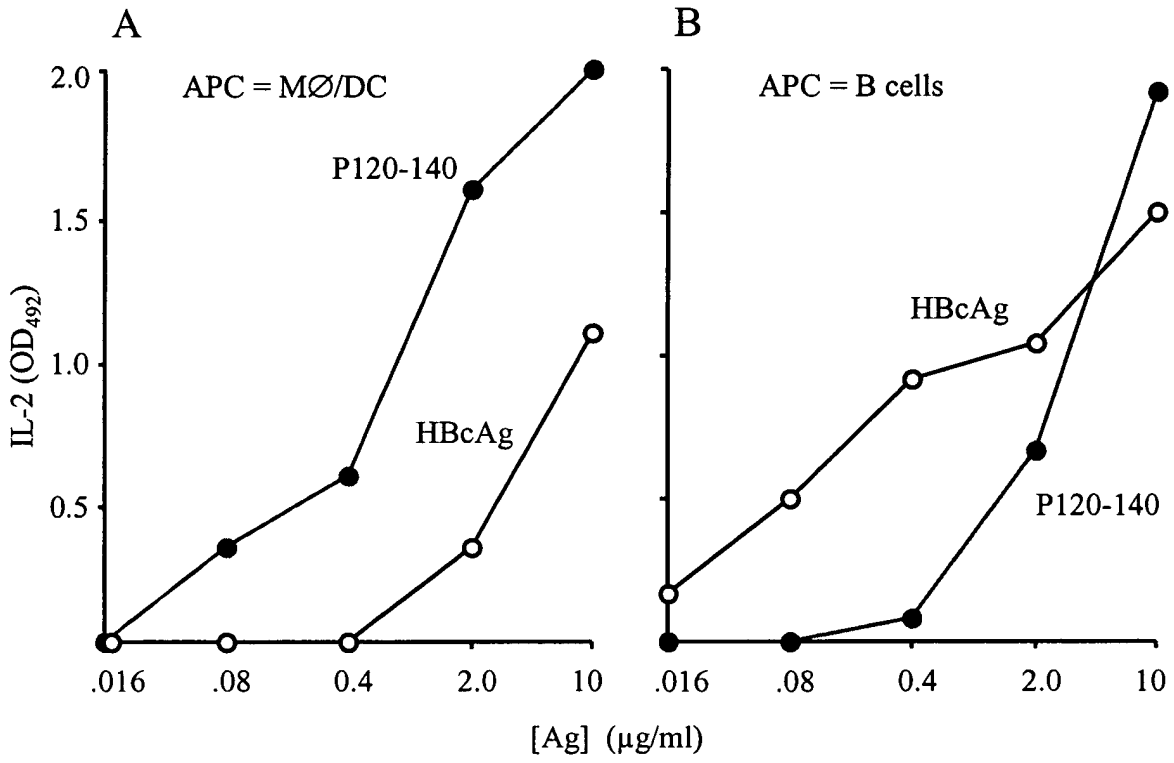

A
B
APC = MØ/DC
APC = B cells
P120-140
HBcAg
HBcAg
P120-140
2.0
1.5
1.0
0.5
IL-2 (OD492)
.016
.08
0.4
2.0
10
[Ag] (μg/ml)

Fig. 24

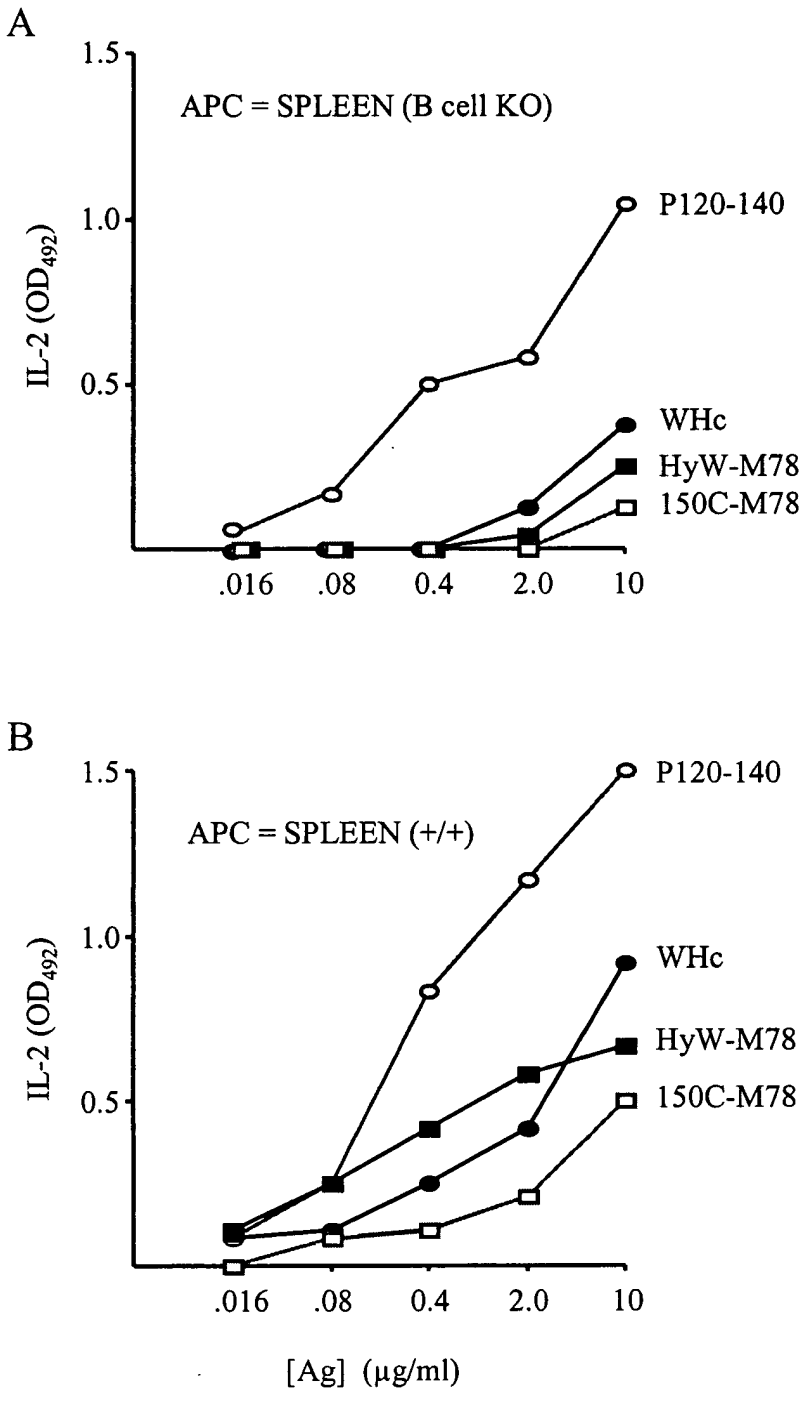

A
1.5
1.0
0.5
IL-2 (OD492)
APC = SPLEEN (B cell KO)
P120-140
WHc
HyW-M78
150C-M78
.016
.08
0.4
2.0
10
B
1.5
1.0
0.5
IL-2 (OD492)
APC = SPLEEN (+/+)
P120-140
WHc
HyW-M78
150C-M78
.016
.08
0.4
2.0
10
[Ag] (µg/ml)

Fig. 25

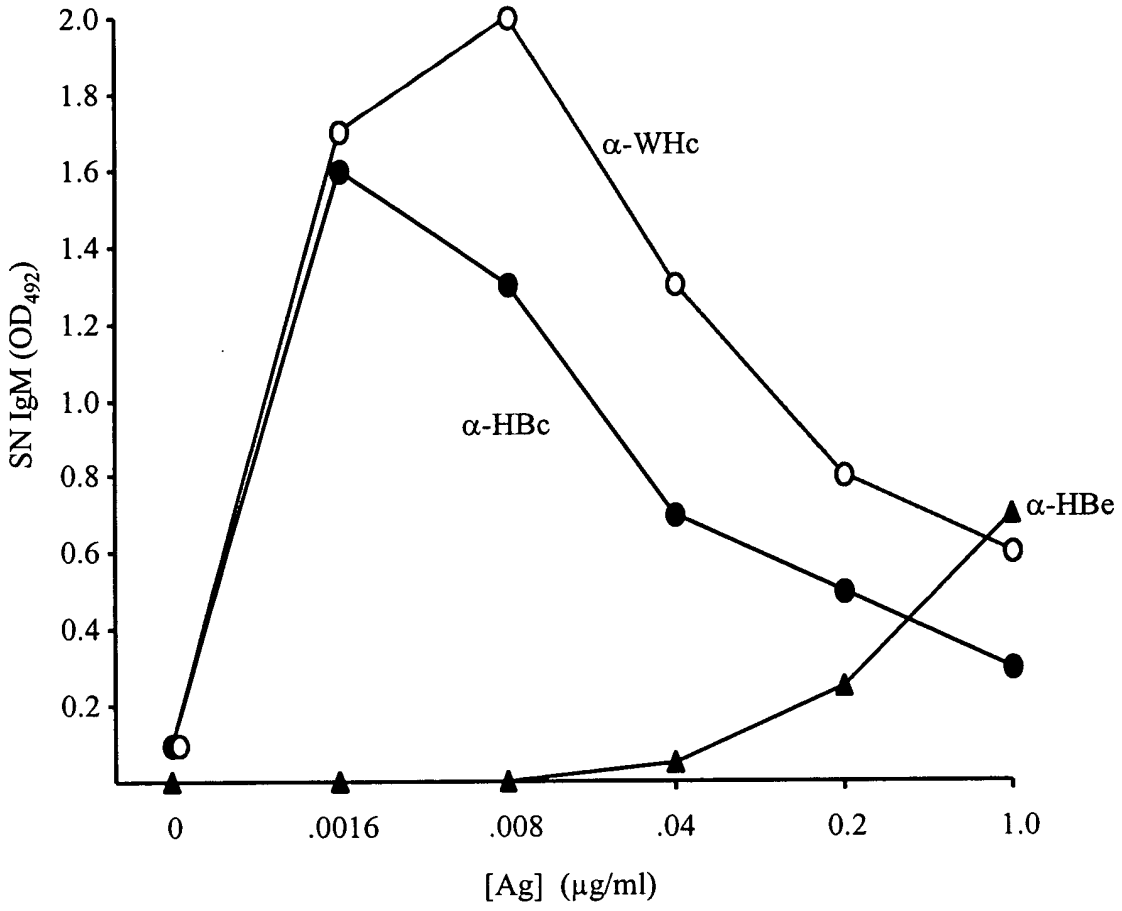

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
SN IgM (OD492)
α-WHc
α-HBc
α-HBe
0
.0016
.008
.04
0.2
1.0
[Ag] (μg/ml)

Fig. 26

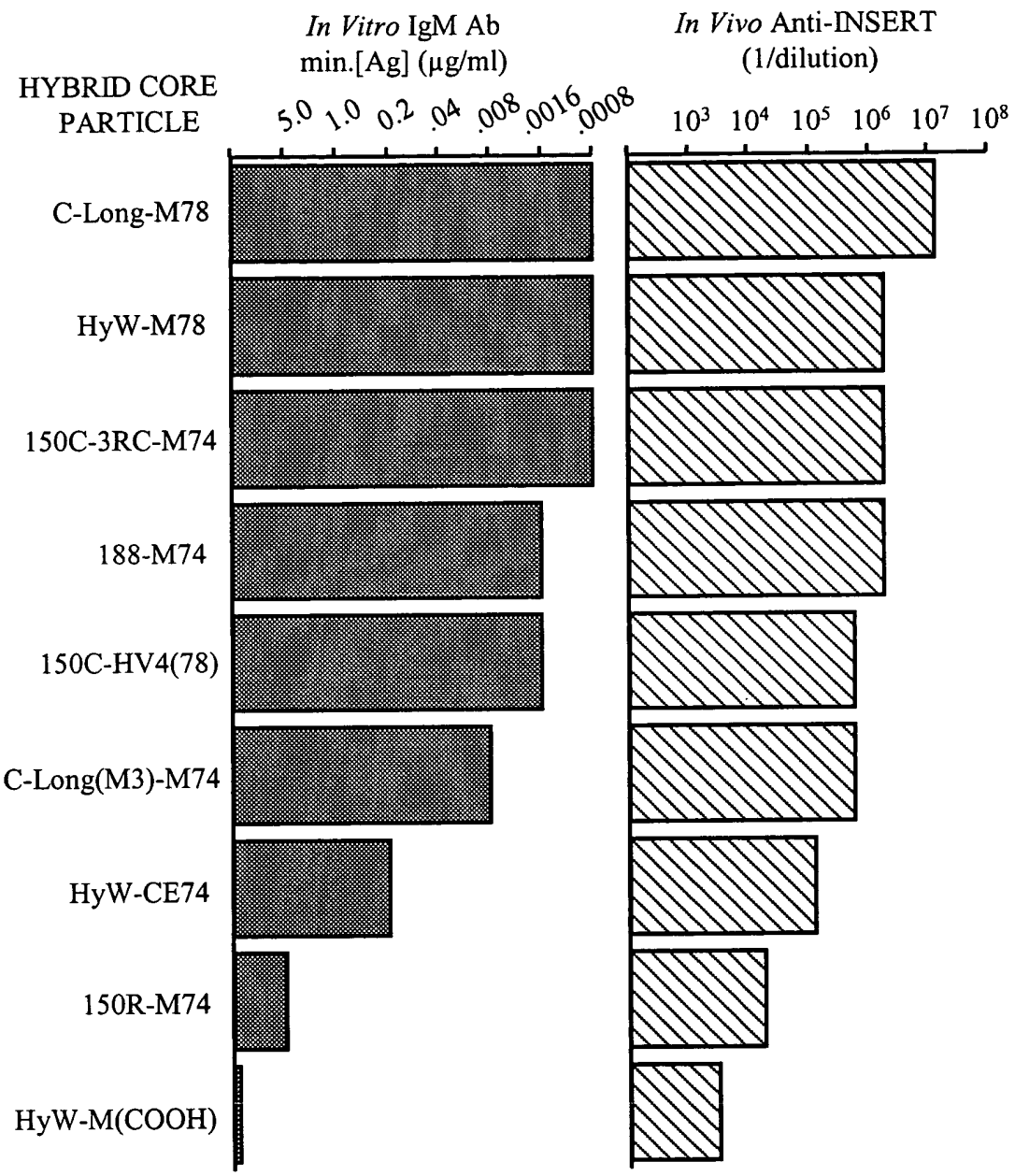

HYBRID CORE PARTICLE
In Vitro IgM Ab min.[Ag] (µg/ml)
5.0 1.0 0.2 .04 .008 .0016 .0008
In Vivo Anti-INSERT (1/dilution)
10^3 10^4 10^5 10^6 10^7 10^8
C-Long-M78
HyW-M78
150C-3RC-M74
188-M74
150C-HV4(78)
C-Long(M3)-M74
HyW-CE74
150R-M74
HyW-M(COOH)

Fig. 27

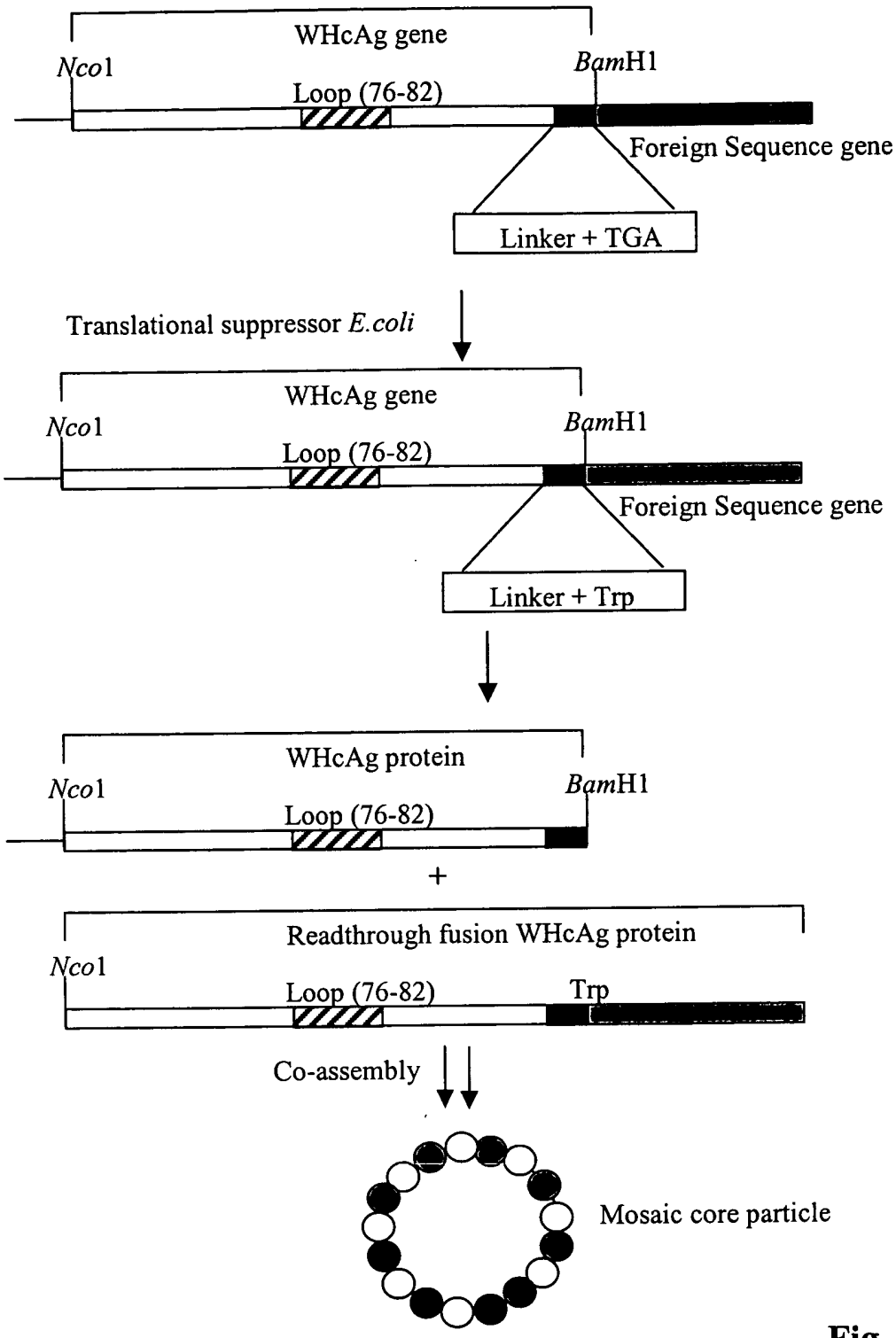

WHcAg gene
NcoI
BamH1
Loop (76-82)
Foreign Sequence gene
Linker + TGA
Translational suppressor E.coli
WHcAg gene
NcoI
BamH1
Loop (76-82)
Foreign Sequence gene
Linker + Trp
WHcAg protein
NcoI
BamH1
Loop (76-82)
+
Readthrough fusion WHcAg protein
NcoI
Loop (76-82)
Trp
Co-assembly
Mosaic core particle

Fig. 28

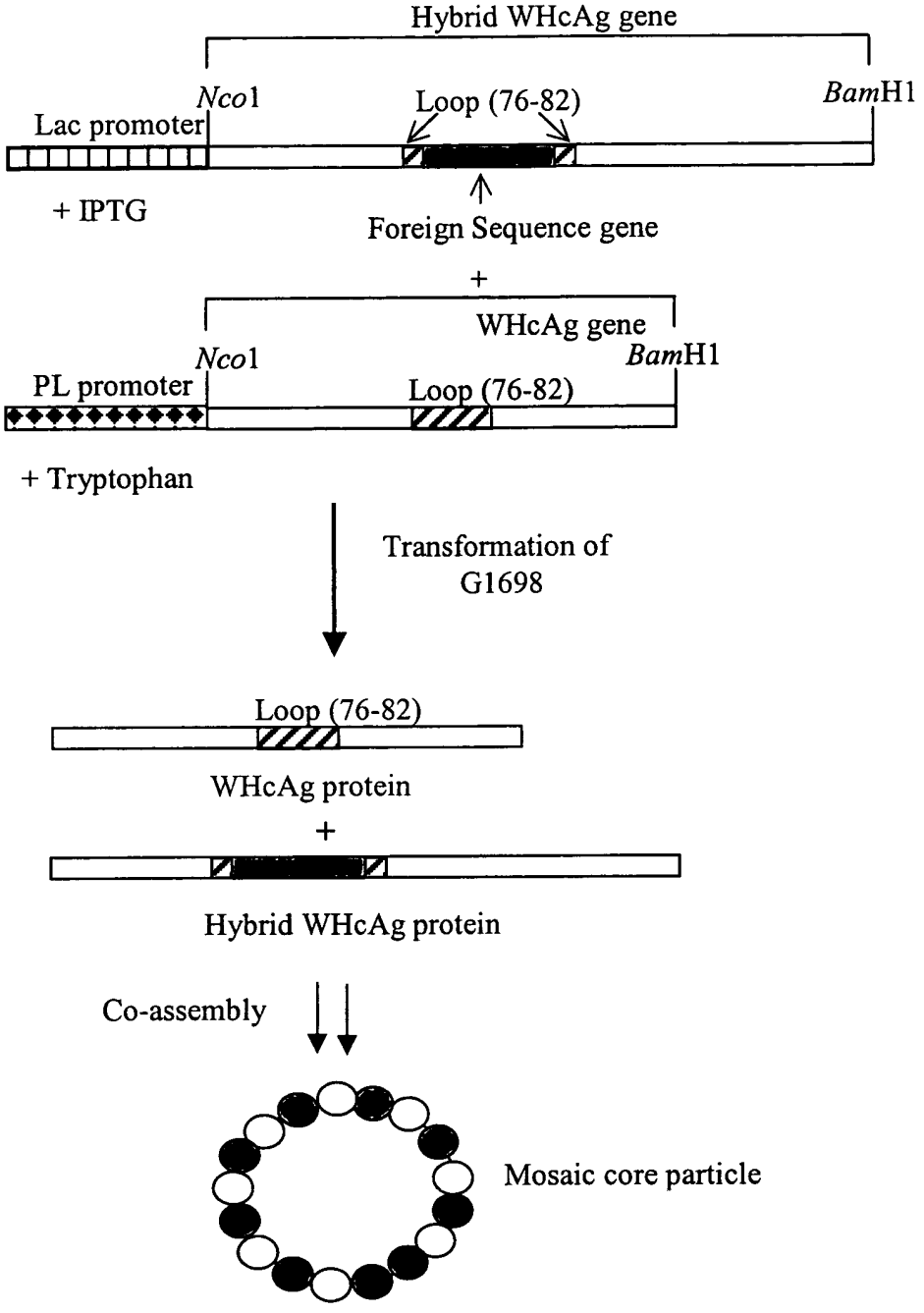

Hybrid WHcAg gene
Nco1
Loop (76-82)
BamH1
Lac promoter
+ IPTG
Foreign Sequence gene
+
WHcAg gene
Nco1
BamH1
PL promoter
Loop (76-82)
+ Tryptophan
Transformation of
G1698
Loop (76-82)
WHcAg protein
+
Hybrid WHcAg protein
Co-assembly
Mosaic core particle

Fig. 29

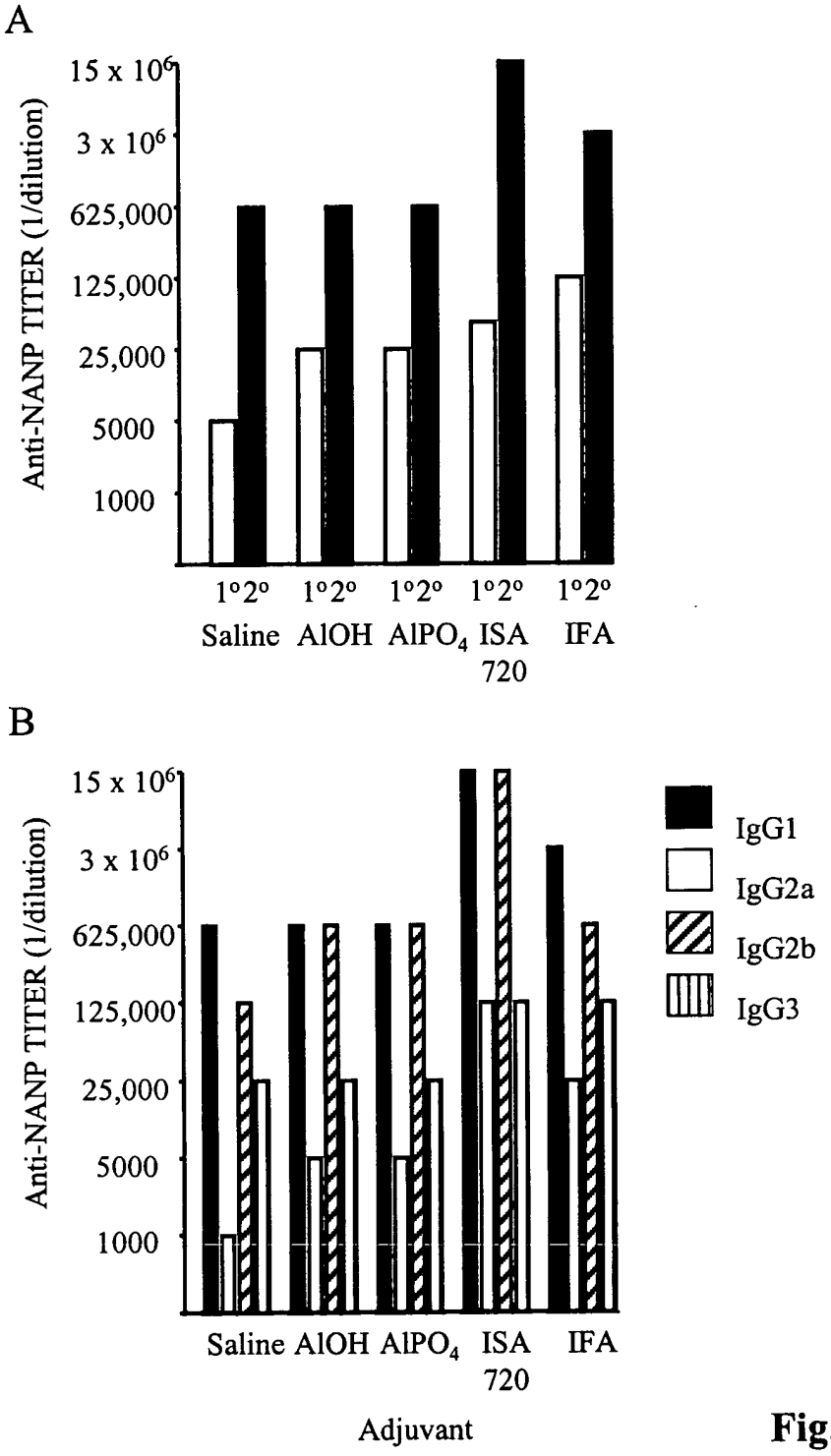

A
15 x 10^6
3 x 10^6
625,000
125,000
25,000
5000
1000
Anti-NANP TITER (1/dilution)
1°2° 1°2° 1°2° 1°2° 1°2°
Saline AlOH AlPO4 ISA 720 IFA
B
15 x 10^6
3 x 10^6
625,000
125,000
25,000
5000
1000
Anti-NANP TITER (1/dilution)
IgG1
IgG2a
IgG2b
IgG3
Saline AlOH AlPO4 ISA 720 IFA
Adjuvant

Fig. 30

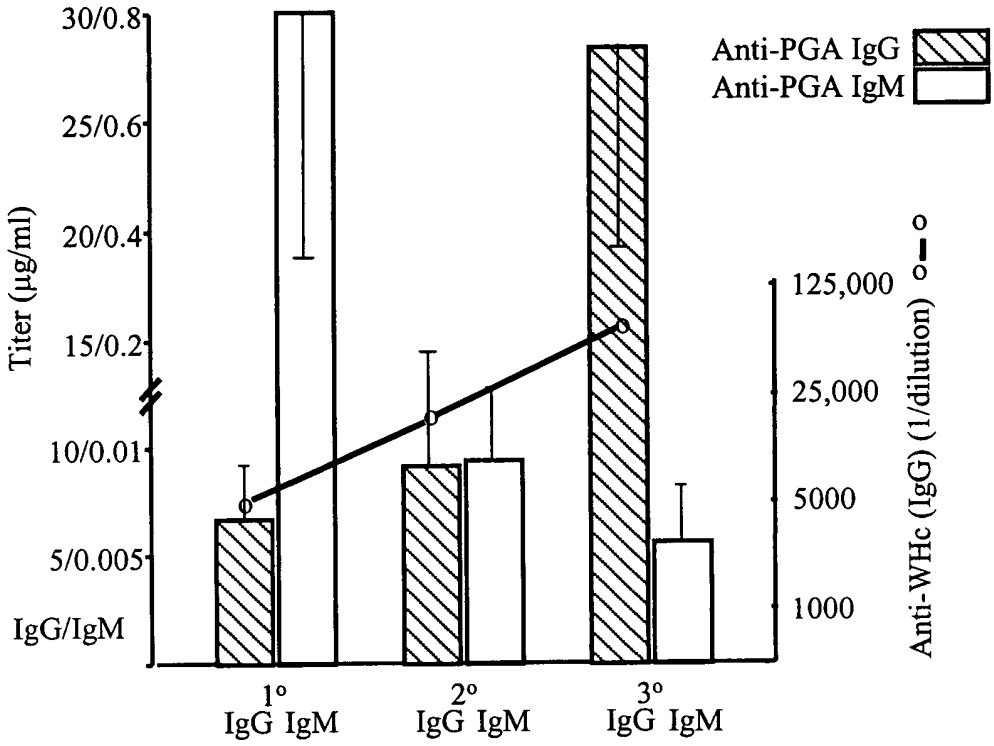

30/0.8
25/0.6
20/0.4
15/0.2
10/0.01
5/0.005
IgG/IgM
Titer (µg/ml)
Anti-PGA IgG
Anti-PGA IgM
125,000
25,000
5000
1000
Anti-WHc (IgG) (1/dilution)
1°
IgG IgM
2°
IgG IgM
3°
IgG IgM

Fig. 31

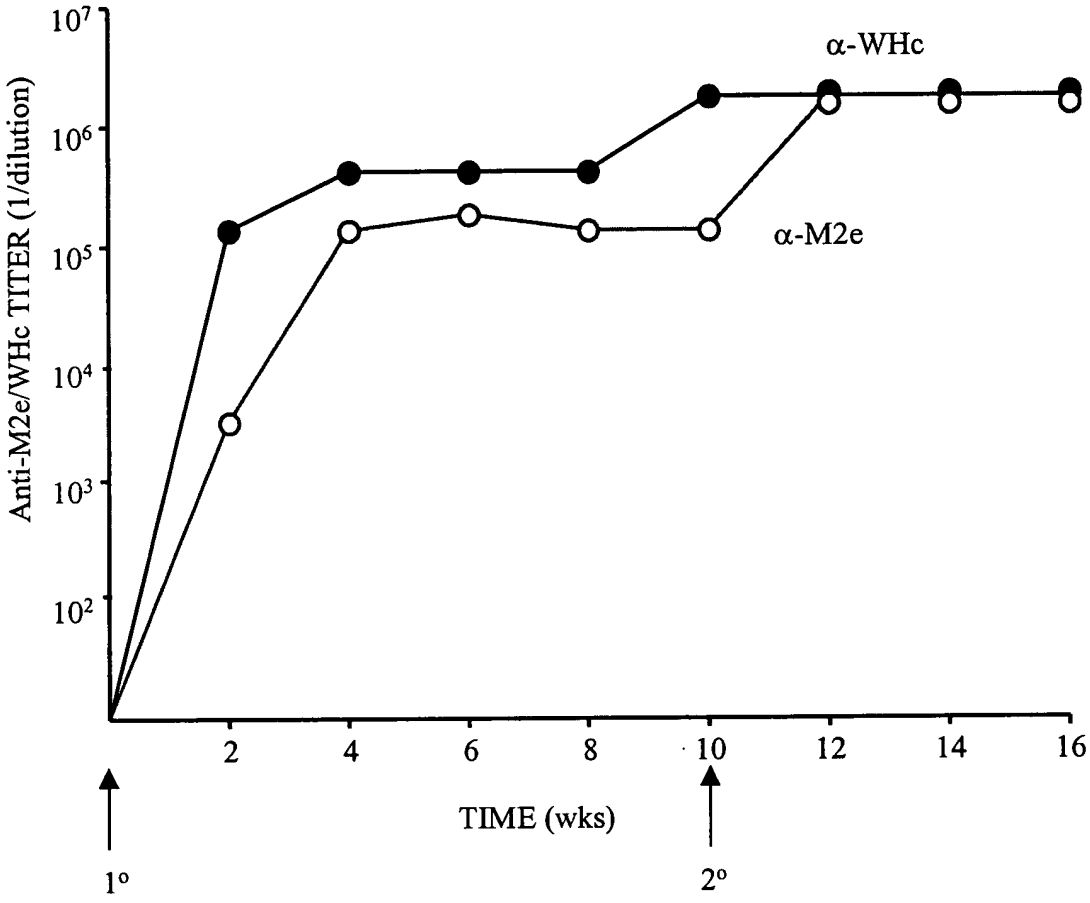

Anti-M2e/WHc TITER (1/dilution)
10^7
10^6
10^5
10^4
10^3
10^2
α-WHc
α-M2e
2
4
6
8
10
12
14
16
TIME (wks)
1°
2°

Fig. 32

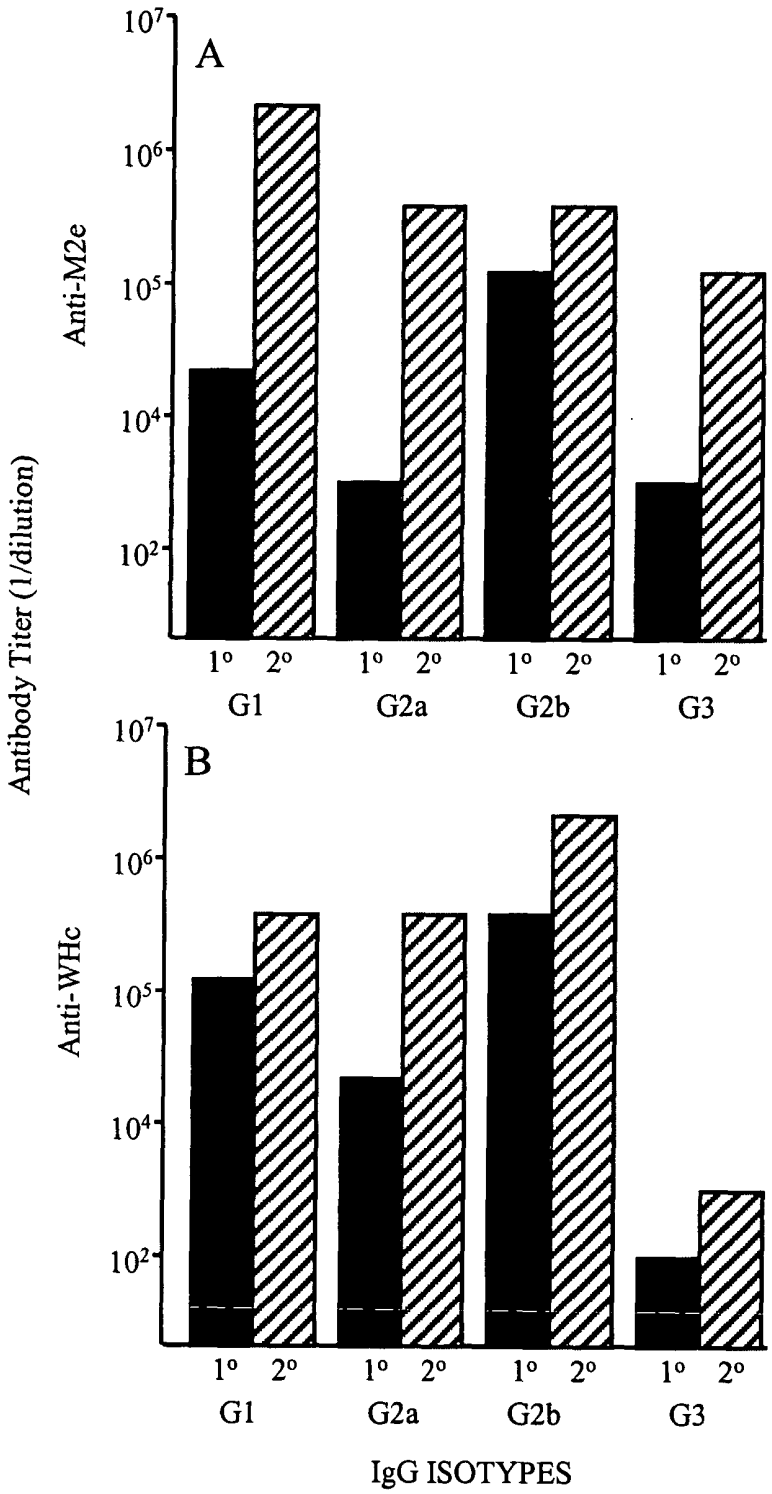

A
B
Anti-M2e
Anti-WHc
Antibody Titer (1/dilution)
10^7
10^6
10^5
10^4
10^2
1° 2°
G1
G2a
G2b
G3
IgG ISOTYPES

Fig. 33

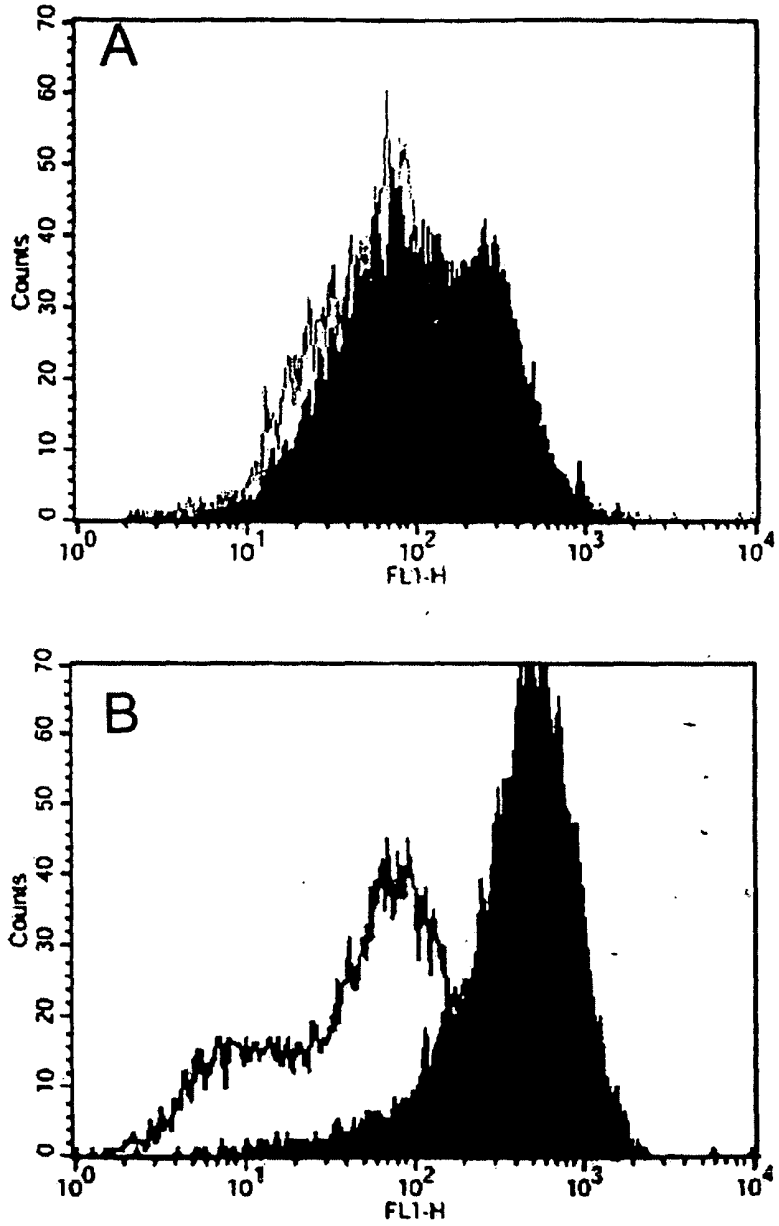


Fig. 34

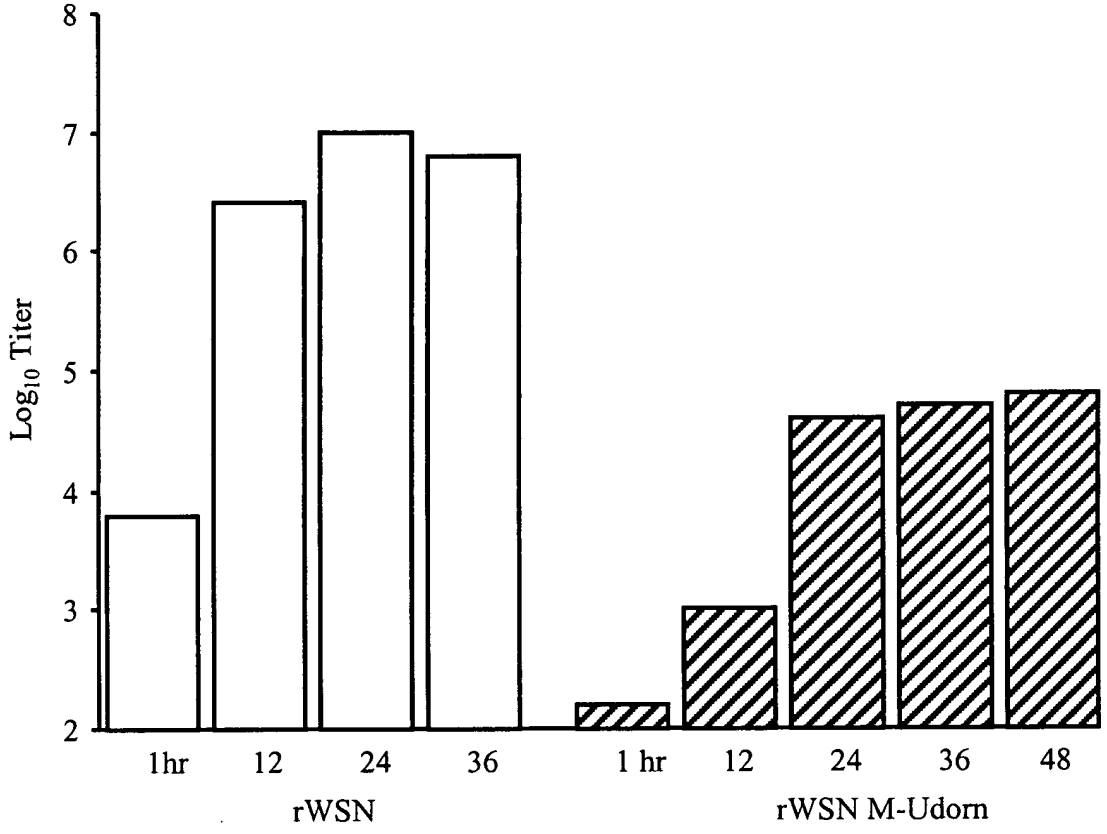

8
7
6
5
4
3
2
Log10 Titer
1hr
12
24
36
1 hr
12
24
36
48
rWSN
rWSN M-Udorn

Fig. 35

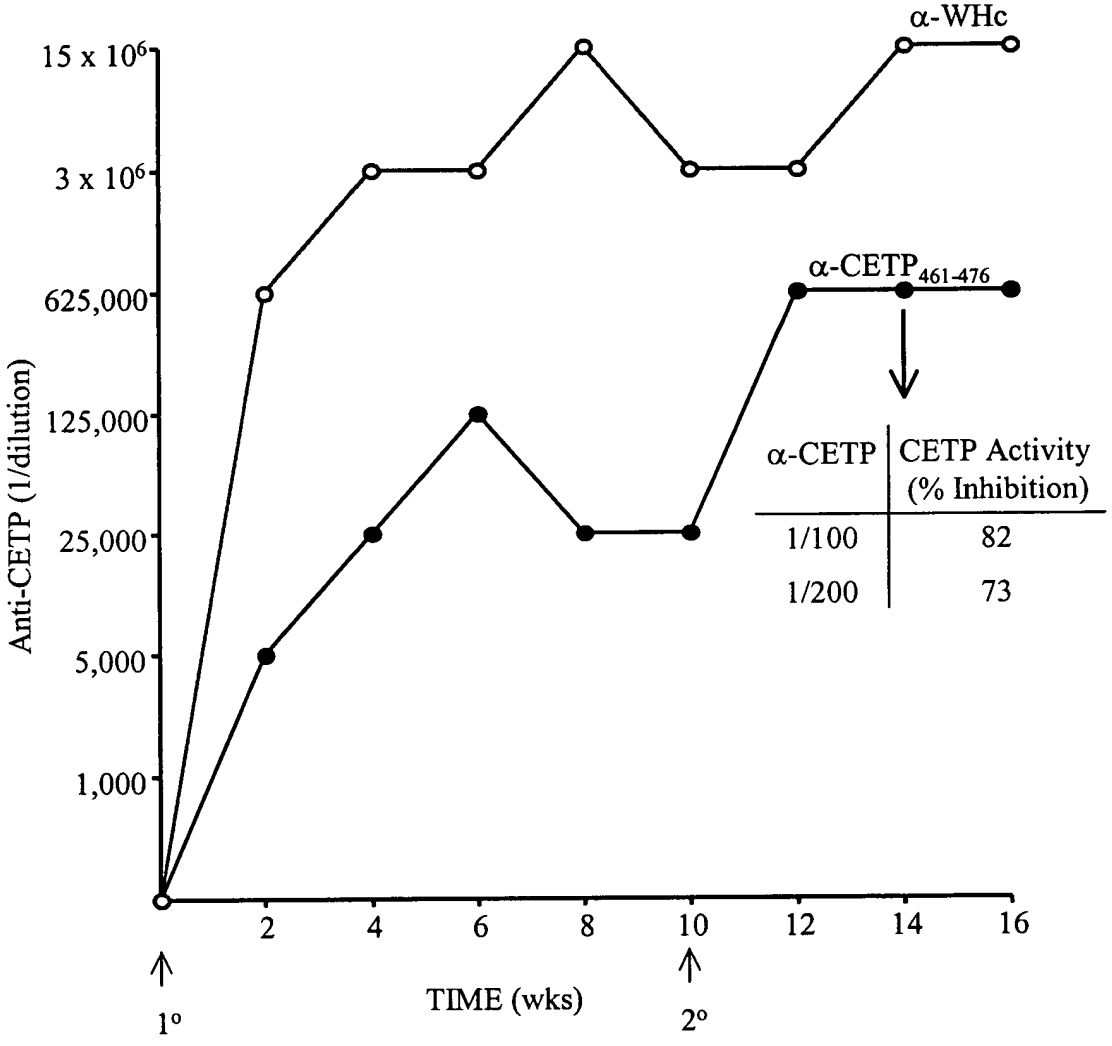

α-WHc
15 x 10^6
3 x 10^6
625,000
125,000
25,000
5,000
1,000
Anti-CETP (1/dilution)
α-CETP461-476
α-CETP
CETP Activity (% Inhibition)
1/100
82
1/200
73
2
4
6
8
10
12
14
16
TIME (wks)
1°
2°

Fig. 36

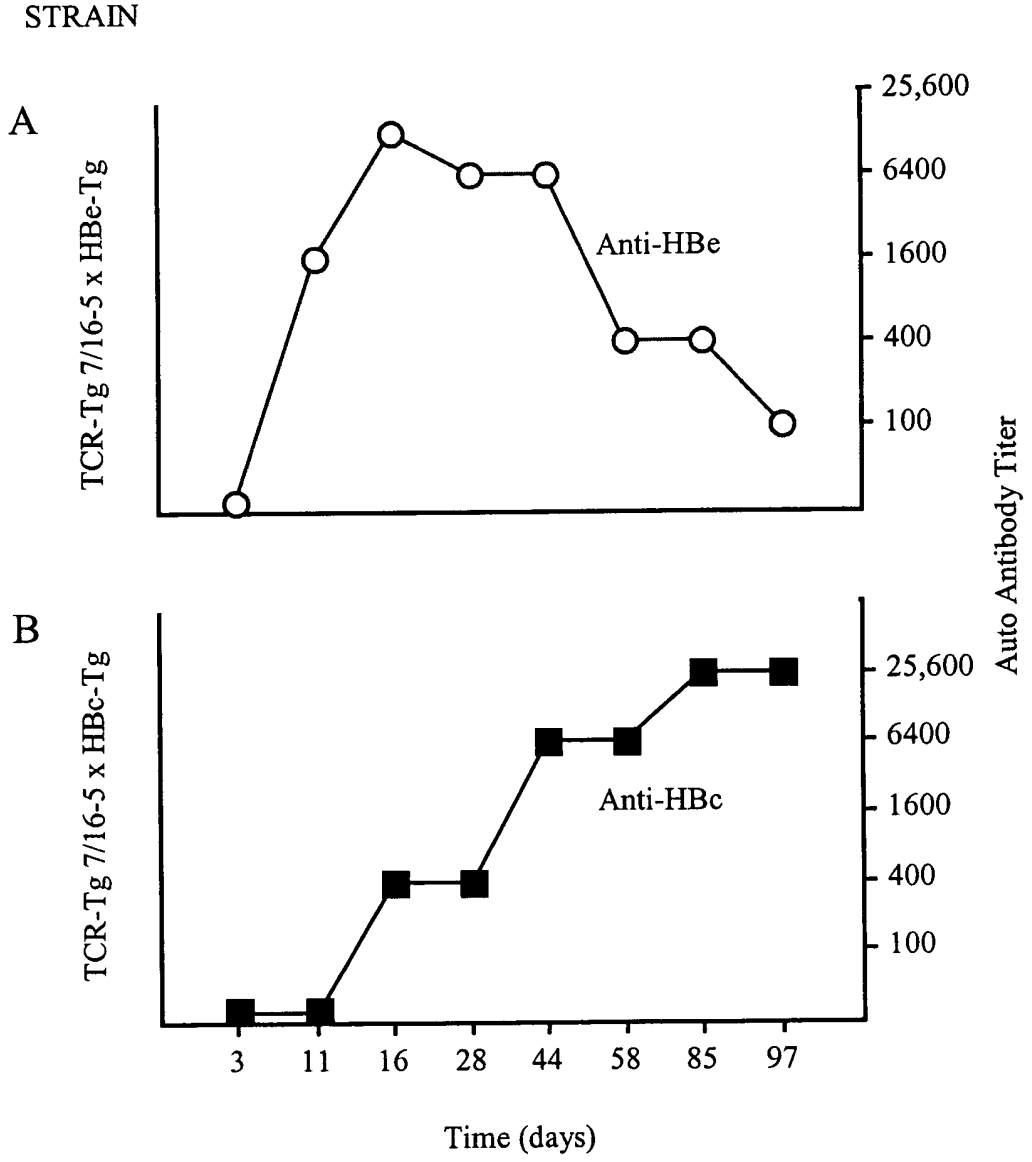

STRAIN
A
TCR-Tg 7/16-5 x HBe-Tg
Anti-HBe
25,600
6400
1600
400
100
B
TCR-Tg 7/16-5 x HBc-Tg
Anti-HBc
25,600
6400
1600
400
100
Auto Antibody Titer
3
11
16
28
44
58
85
97
Time (days)

Fig. 37

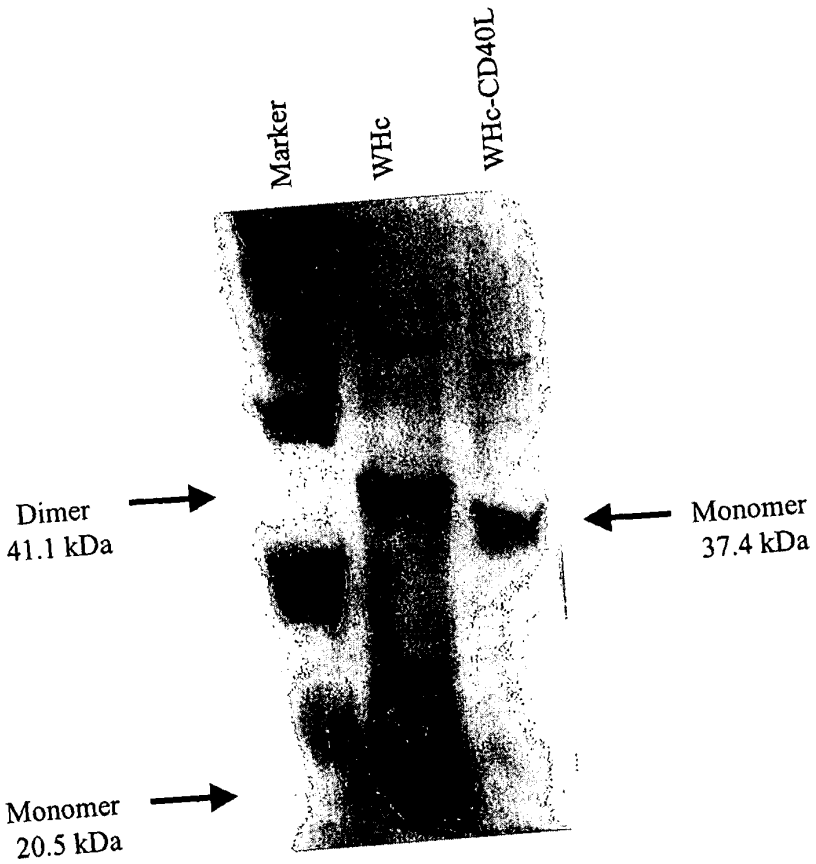

Marker
WHc
WHc-CD40L
Dimer
41.1 kDa
Monomer
37.4 kDa
Monomer
20.5 kDa

Fig. 38

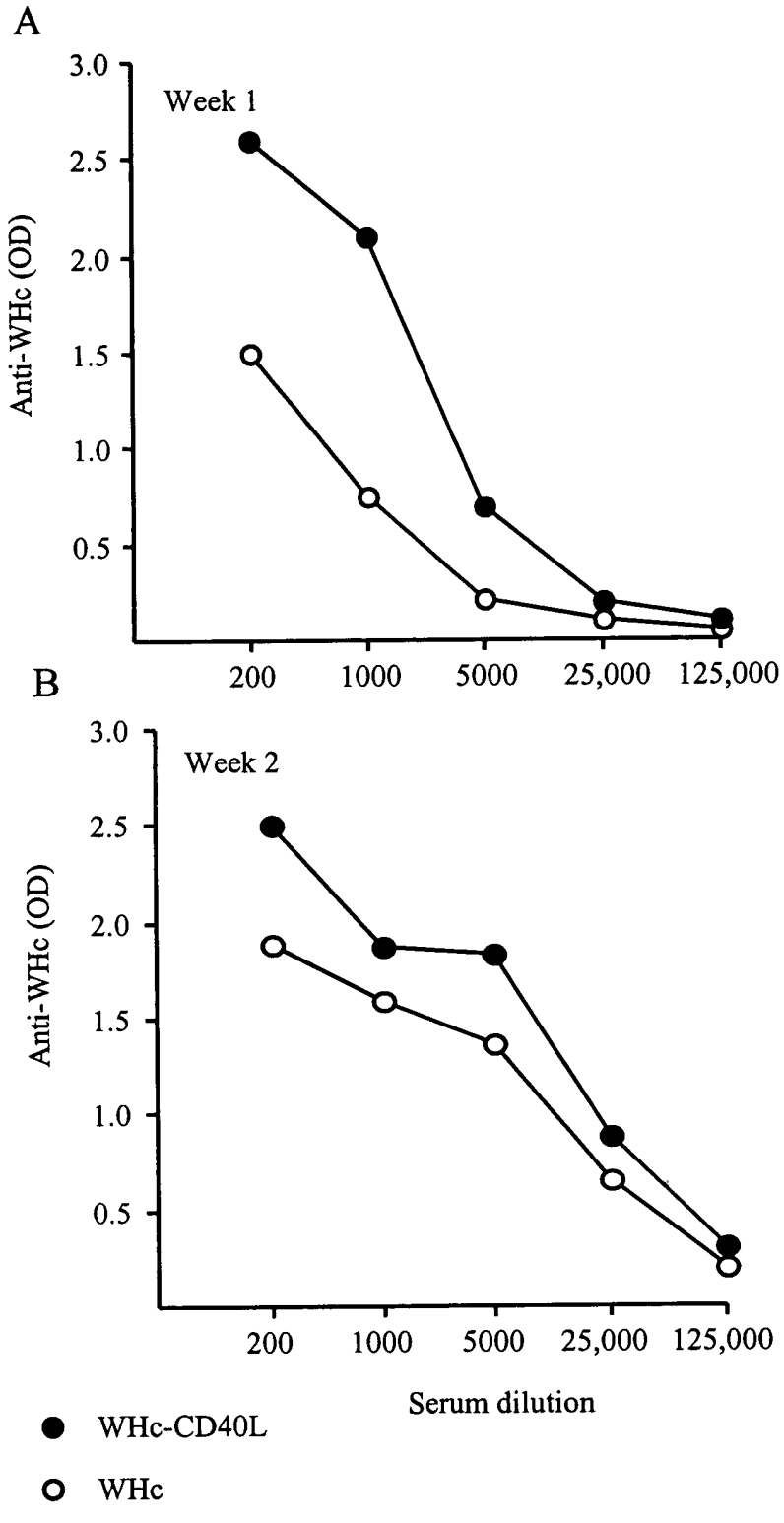

A
3.0
2.5
2.0
1.5
1.0
0.5
Anti-WHc (OD)
Week 1
200
1000
5000
25,000
125,000
B
3.0
2.5
2.0
1.5
1.0
0.5
Anti-WHc (OD)
Week 2
200
1000
5000
25,000
125,000
Serum dilution
WHc-CD40L
WHc

Fig. 39

Fig. 40

A Wild Type WHcAg DNA (SEQ ID NO:37)

ATGGACATAGATCCCTATAAAGAATTTGGTTCATCTTATCAGTTGTTGAATTTTCTTCC
TTTGGACTTCTTTCCTGACCTTAATGCTTTGGTGGACACTGCTACTGCCTTGTATGAAG
AAGAGCTAACAGGTAGGGAACATTGCTCTCCGCACCATACAGCTATTAGACAAGCTTTA
GTATGCTGGGATGAATTAACTAAATTGATAGCTTGGATGAGCTCTAACATAACTTCTGA
ACAAGTAAGAACAATCATTGTAAATCATGTCAATGATACCTGGGGACTTAAGGTGAGAC
AAAGTTTATGGTTTCATTTGTCATGTCTCACTTTCGGACAACATACAGTTCAAGAATTT
TTAGTAAGTTTTGGAGTATGGATCAGGACTCCAGCTCCATATAGACCTCCTAATGCACC
CATTCTCTCGACTCTTCCGGAACATACAGTCATTAGGAGAAGAGGAGGTGCAAGAGCTT
CTAGGTCCCCCAGAAGACGCACTCCCTCTCCTCGCAGGAGAAGATCTCAATCACCGCGT
CGCAGACGCTCTCAATCTCCATCTGCCAACTGCTGA

B Wild Type WHcAg (SEQ ID NO:1)

MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQAL
VCWDELTKLIAWMSSNITSEQVRTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEF
LVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPR
RRRSQSPSANC

C Truncated WHcAg (SEQ ID NO:38)

MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQAL
VCWDELTKLIAWMSSNITSEQVRTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEF
LVSFGVWIRTPAPYRPPNAPILSTLPEHTVI

Fig. 41

A Wild Type GSHcAg DNA (SEQ ID NO:39)

ATGGACATAGATCCCTATAAAGAATTTGGTTCTTCTTATCAGTTGTTGAATTTTCTTCC
TTTGGACTTTTTTCCTGATCTCAATGCATTGGTGGACACTGCTGCTGCTCTTTATGAAG
AAGAATTAACAGGTAGGGAGCATTGTTCTCCTCATCATACTGCTATTAGACAGGCCTTA
GTGTGTTGGGAAGAATTAACTAGATTAATTACATGGATGAGTGAAAATACAACAGAAGA
AGTTAGAAGAATTATTGTTGATCATGTCAATAATACTTGGGGACTTAAAGTAAGACAGA
CTTTATGGTTTCATTTATCATGTCTTACTTTTGGACAACACACAGTTCAAGAATTTTTG
GTTAGTTTTGGAGTATGGATTAGAACTCCAGCTCCTTATAGACCACCTAATGCACCCAT
TTTATCAACTCTTCCGGAACATACAGTCATTAGGAGAAGAGGAGGTTCAAGAGCTGCTA
GGTCCCCCCGAAGACGCACTCCCTCTCCTCGCAGGAGAAGGTCTCAATCACCGCGTCGC
AGACGCTCTCAATCTCCAGCTTCCAACTGCTGA

B Wild Type GSHcAg (SEQ ID NO:21)

MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTAAALYEEELTGREHCSPHHTAIRQAL
VCWEELTRLITWMSENTTEEVRRIIVDHVNNTWGLKVRQTLWFHLSCLTFGQHTVQEFL
VSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGSRAARSPRRRTPSPRRRRSQSPRR
RRSQSPASNC

C Truncated GSHcAg (SEQ ID NO:40)

MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTAAALYEEELTGREHCSPHHTAIRQAL
VCWEELTRLITWMSENTTEEVRRIIVDHVNNTWGLKVRQTLWFHLSCLTFGQHTVQEFL
VSFGVWIRTPAPYRPPNAPILSTLPEHTVI

Fig. 42

A Wild Type HBcAg DNA (SEQ ID NO:57)

ATGGACATCGACCCTTATAAAGAATTTGGAGCTACTGTGGAGTTACTCTCGTTTTTGCC
TTCTGACTTCTTTCCTTCAGTACGAGATCTTCTAGATACCGCCTCAGCTCTGTATCGGG
AAGCCTTAGAGTCTCCTGAGCATTGTTCACCTCACCATACTGCACTCAGGCAAGCAATT
CTTTGCTGGGGGGAACTAATGACTCTAGCTACCTGGGTGGGTGTTAATTTGGAAGATCC
AGCATCCAGAGACCTAGTAGTCAGTTATGTCAACACTAATATGGGCCTAAAGTTCAGGC
AACTCTTGTGGTTTCACATTTCTTGTCTCACTTTTGGAAGAGAAACCGTTATAGAGTAT
TTGGTGTCTTTCGGAGTGTGGATTCGCACTCCTCCAGCTTATAGACCACCAAATGCCCC
TATCCTATCAACACTTCCGGAAACTACTGTTGTTAGACGACGAGGCAGGTCCCCTAGAA
GAAGAACTCCCTCGCCTCGCAGACGAAGGTCTCAATCGCCGCGTCGCAGAAGATCTCAA
TCTCGGGAATCTCAATGTTGA

B Wild Type HBcAg (SEQ ID NO:41)

MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAI
LCWGELMTLATWVGVNLEDPASRDLVVSYVNTNMGLKFRQLLWFHISCLTFGRETVIEY
LVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQ
SRESQC

C Truncated HBcAg (SEQ ID NO:58)

MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAI
LCWGELMTLATWVGVNLEDPASRDLVVSYVNTNMGLKFRQLLWFHISCLTFGRETVIEY
LVSFGVWIRTPPAYRPPNAPILSTLPETTVV